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MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XLV JANUARY–FEBRUARY, 1953 No. 1

THE PURE CULTURE IN TAXONOMY[1]

JOSEPH C. GILMAN[2]

(WITH PORTRAIT)

In 1881 Lankester, commenting on the announcement of Koch at the International Medical Congress in London that year, of his use of the gelatin plate for culturing bacteria, made the following statement:

"It is only by such monosporous cultivations that we can arrive at solid conclusions in reference to the forms and activities of the bacteria, e.g. as to whether one form can give rise to progeny of another form when its food and conditions of growth are changed, and again, as to whether special fermentative powers can be lost or acquired in the course of generations derived from one parent germ, but subjected to different conditions as to food, temperature and oxygen."

While this statement was concerned with the bacteria its implications in respect to fungi are obvious.

From the standpoint of techniques, Koch's use of gelatin culture was the turning point in laboratory practice in both bacteriology and mycology. It is true that pure cultures had been obtained earlier by dilution methods as devised by Pasteur and Lister. These

[1] Journal paper No. J- 3 of the Agricultural Experiment Station, Ames, Iowa. Project No. 101 .

[2] Presidential address, Mycological Society of America. Presented September 8, 1952, at the Society meetings in Ithaca, N. Y.

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methods were cumbersome and laborious and the results therefrom were uncertain. When the investigator could combine the dilution series with subsequent growth on a solid medium, with the single cells fixed in space on the surface, his results were clear and he could proceed to further experiment with confidence.

Culturing of micro-organisms, fungi and bacteria was not new. Mycologists like Brefeld and DeBary had cultured their fungi many years before, but they worked largely in the same manner as a gardener, growing their organisms in spite of contamination. Pasteur and Lister were using solid media—such materials as potato plugs that were opaque and not amenable to the critical examination made possible by the transparent background of the gelatin film.

Soon Frau Hesse, working in Koch's laboratory, announced the use of agar-agar as the base for culture-media and the foundations for modern culture techniques were laid. From then on, this tool was available and the work of adapting it to its many modern uses and keeping it sharp for critical study has been the labor of mycologists, bacteriologists and biochemists. It is my purpose to examine the record of the seventy years that have passed and discuss some of the advances made possible by the use of this tool. How far have Lankester's predictions been confirmed?

His "solid conclusions" were to be found in reference to the "forms" of the organisms and to the "activities" of them. In other words, how far have we advanced in our knowledge of morphology of the fungi and with it of the taxonomy that is based on such morphology and what advances have been made in our knowledge of fungous physiology? Let us examine these two fields separately, discussing the latter first.

In the field of physiology there have been tremendous advances, particularly in regard to the substrate, and from that in regard to the nutritional needs of many fungi. We might divide our discussion into three parts: mineral nutrition, vitamins and antibiotics.

By means of the pure culture the effects of minerals, vitamins, sugars and proteins on the growth and sporulation of fungi have been extensively and intensely studied. Steinberg, for example, has shown that amounts of particular mineral elements far below the level measurable by the ordinary chemical balance affect the

growth rate, coloration and sporulation of fungi to a degree that is readily recognizable by gross examination. Similar studies on the vitamin requirements of fungi in culture have been demonstrated by Robbins and Lilly and their associates. On the other hand Raper and Whiffen have demonstrated the extreme variability present in pure cultures when single spore isolates from a single colony of a fungus were plated and the progeny grown to maturity. A somewhat similar variation, in virulence rather than morphologic characteristics, was encountered when strains of *Phytophthora infestans* from potato and tomato were grown from single zoospores and the progeny compared with regard to their pathogenicity. Examples of this kind might be multiplied indefinitely. Mass transfers, in contrast, tend to maintain a uniform type of colony. Such reactions are most readily explained by postulating a wide variability in the genetic composition of the mycelial nuclei in any particular colony of the fungus. In these studies the uniformity of substratum on which the progeny was grown was unquestionably accepted in the first case or, in the case of pathogenicity studies, distinctions, such as tomato plants and potato plants, were clearly defined. However, when fungi are isolated and studied in different laboratories or even at different times in the same laboratory, this variability might well account for multiplicity of species from either single spore cultures or the selectivity of the medium used for a particular study.

Granted that great genetic variability is present in most fungous thalli, if we accept the inadequacies of the chemical balance to measure biological quantities of reagents and the lack of biological purity in most of the commercial supplies of the commonly used chemicals as well as chemical contaminants in any but especially prepared glassware, should we not expect biological variants to appear regularly in our cultures?

To cloud the issue further, the needs of fungi for growth and sporulation are varied with respect to vitamin requirements and hormonal action. Not only do these requirements differ from species to species but many of them vary genetically for strains, clones, or individual thalli within a species. Hence these needs must be supplied either by supplementing the medium with one or more constituents or the medium itself should contain them, and

the investigator is forced to use natural substrata, cooked stems, leaves or other plant or animal parts for the growth of his fungi. It might be argued that these natural substrata, if composed of similar parts of similar plants, for example, are more biologically constant than two samples of any synthetic medium. Whether this be the case or not, such natural substrata have been found in practice to be the most available means of meeting the needs of the investigator who required an abundant supply of spores or an ascus stage to carry out his study. In their studies on *Fusarium*, Appel and Wollenweber recommended cooked stems for the production of their "normal" conidia, to name one of the earlier instances of a swing from synthetic to natural media.

From pure culture studies we have a great mass of information concerning the nutritional needs of individual species. As yet no general pattern of behavior is apparent either in respect to physical and chemical responses to environment, or to taxonomic units. Such a situation is probably to be expected with a group as large and diverse as the fungi, yet we are continually trying to find some further orderliness in our taxonomic structure.

Pure cultures have provided an expanding field of research in relation to the production of antibiotics by fungi but with the excellent synopsis by Raper last year, this phase needs no discussion at this time.

Taxonomically the pure culture has provided the basis for great advances. Of primary importance was the connecting of different stages in the cycles of the fungi, making it possible to demonstrate on an experimental basis the connections that had been postulated previously from association and sequence of appearance so ably observed by the Tulasne brothers. With the pure culture came the knowledge of heterothallism, that needs only mention, since it has been ably discussed so recently by Whitehouse. In plant pathology it led to Koch's postulates and to a clearer understanding of parasitism; with the latter came a re-evaluation of the taxonomy of the Imperfecti. Cross-inoculations have shown that many species that were earlier set up on a host basis are not confined to a narrow range of host plants; much synonymy has resulted. This situation unfortunately does not simplify the problem, for many of

the species have common hosts and inoculation trials need be carried out to separate the species involved.

From the point of view of taxonomy the use of pure cultures has given rise to two schools of thought. While I am not sufficiently optimistic to think that anything I may say will bring in an unanimous opinion reconciling these points of view, I wish to examine them somewhat critically as a means of clarifying our thinking. On the one hand we find those investigators who emphasize the fungus and look for a medium for culturing it that will give optimum conditions for its growth and sporulation. Such a medium under proper temperature, moisture, or other environmental conditions should supply the necessary vitamins, minerals, proteins and carbohydrates for completion of the cycle in culture. No universal single medium would serve since we know that the requirements of a given species differ markedly from those of many other species, and also vary with the age of the culture, the length of time the fungus has been separated from its natural substrata, and the degree of specialization toward its substrata that the fungus has acquired. The gamut of conditions is almost infinite, and the crux of the problem still lies within the judgment of the investigator to determine the evasive ideal of what is optimum, or what is normal. The difficulties of making such a judgment have led to the opposing idea: the selection of a single medium on which the growth of the fungi under investigation would be compared and their responses noted. Here the selection of the proper medium becomes paramount. Certain workers have advocated a purely synthetic medium such as Czapek's or a modification of it. Unfortunately such media are definitely limited in their usefulness when the range of cultivable fungi is taken into account. The saprobic molds can be treated in this manner very successfully but the facultative parasites, many of which are serious plant pathogens, do not lend themselves to such treatment, principally because many of them are unable to synthesize the growth substances necessary for fruition or for growth itself. Further criticism of such a procedure might be raised; that in the light of Steinberg's findings, we do not have the implementation necessary to insure sufficiently accurate measurements of ingredients to be confident that any two batches of medium are biologically identical. The sensitivity of the fungus is so great

that it defeats the validity of any comparisons based upon a single medium. Another difficulty in the use of synthetic media arises from the fact that many of the fungi, particularly acervulate and sporodochial forms, seemingly need a particular substrate structure for the formation of such fruits. The sporodochial structure frequently is difficult to demonstrate on agar cultures.

Faced with the above dilemma, the investigator will still find it necessary to accept the situation and govern his procedures by the fungus involved. The pure culture has proved a potent tool but its use still must be mastered. It has taught us much concerning the physiology and genetics of fungi and has been extremely useful in studies of parasitism and the variability of fungi. Taxonomically it still must be used with care, particularly in regard to the Fungi Imperfecti, a group whose taxonomy was so strikingly commented upon by the late Dr. J. J. Davis, whom many of our older members, I am sure, will remember. His comment was:

"You will find a fungus and determine its characteristics. You turn to the books and decide on its genus. Then you look for the species. And you look, and you look, and after a while you find it! In another genus!"

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COPROGEN, A NEW GROWTH FACTOR PRESENT IN DUNG, REQUIRED BY PILOBOLUS SPECIES

C. W. Hesseltine, A. R. Whitehill, C. Pidacks, Margaret Ten Hagen, N. Bohonos, B. L. Hutchings, and J. H. Williams

(with 8 figures)

There are very few references in the literature (**1**, **2**, **3**, **4**, **5**, **7**, **8**, **9**, **12**) dealing with the cultivation in pure culture of species of *Pilobolus*. In these reported cases of pure culture, a dung decoction has always been a necessary constituent of the media used for growth. This fact seemed rather remarkable considering the extensive literature on the morphology, light relationships, spore discharge, etc. of this genus. It seemed of interest to secure an isolate of one of these species of *Pilobolus* and to determine its growth requirements. If an unknown growth factor were present, it would be of considerable interest to isolate this compound for further biological evaluation.

ISOLATION AND MAINTENANCE OF CULTURE

In January, 1950, several collections of fresh horse dung obtained from a pasture on the grounds of Lederle Laboratories at Pearl River, New York, were brought into the laboratory and placed in glass dishes to which a few ml. of tap water had previously been added. The dishes were covered to bring about a saturated atmosphere, and then they were placed in a north window. These gross cultures were allowed to stand for several days at room temperature. In making initial isolations it is imperative that only a small amount of water be added to each dish to keep the surrounding atmosphere saturated. Too much water causes water-soaking of the dung balls, leading to excessive multiplication of bacteria, which is unfavorable for *Pilobolus* growth. On the fourth day a great abundance of *Pilobolus* sporangiophores appeared. Al-

though the length of the sporangiophores varied considerably, it was apparent that only one species was present. Successive crops of sporangiophores were produced over a period of several days.

Since mature sporangia are shot away from the sporangiophores towards the light source, they could be caught on a poured agar Petri dish by inverting the bottom of the plate between the sporangiophores and the light. Sporangia are typically discharged each morning. In this manner many sporangia were collected on the surface of dung agar. Spore germination was accomplished by placing a single sporangium in a hanging drop to which a commercial preparation of pancreatin had been added. The germinating spores were then streaked out on dung agar in a Petri dish. After several hyphal-tip transfers, the *Pilobolus* was freed of contaminating bacteria and fungi. It was observed in several cases that a bacterium seemed to stimulate the production of trophocysts.

The *Pilobolus* species, when isolated, showed mycelial growth just below the surface of the agar. No aerial mycelium is ever formed except for the transitory sporangiophores which collapse at the time of sporangial discharge. Fruiting is characteristically around the margin of the Petri dish as if exhaustion of a nutrient brought about spore production. However, fruiting may occur over the entire surface of the plate. Fruiting is preceded by the formation of trophocysts, which are cut off by a septum from the coenocytic mycelium and can be used as indicators of the time when sporangial formation is about to occur. Microscopically the trophocysts are yellow and turnip-shaped. The sporangiophores are variable in height. The sporangia, at the time of discharge, are black, smooth-walled, without a reticulate sporangial wall, and the majority measure 148–220 μ in diameter. The sporangiospores are imbedded in a gelatinous matrix in the sporangia making their separation difficult. The sporangiospores from freshly discharged sporangia are ovoid, yellow, thin-walled, and measure 11–16 × 6–9.8 μ. From the key in Buller (**4**) and in Hesseltine (**6**) this *Pilobolus* species is *Pilobolus kleinii* van Tieghem.

Two stock solutions were used in the culture medium.

I. Dung decoction was prepared by suspending 125 g. of Bovung [1] (a commercial dried cow manure) in 1000 ml. of tap water for 2

[1] Walker-Gordon Laboratory Co.

to 3 hours and successively filtering through 2 thicknesses of cotton gauze and filter paper.

II. Oatmeal extract was prepared by boiling a 2 per cent oatmeal in tap water for five minutes and straining the hot solution through 2 thicknesses of cotton gauze.

To prepare the medium, 15 g. of crude agar were added to a mixture of 500 ml. of dung decoction and 500 ml. of oatmeal extract, and this was autoclaved for 15 minutes at 15 lbs. pressure. It is important not to overautoclave this solution. The above dung agar is referred to as the stock culture medium or as dung agar, and was used routinely throughout the work. It was also possible to grow *Pilobolus* cultures on McVickar's dung agar (**9**) and also on asparagine dextrose agar to which some of the above dung decoction had been added. These latter two media have the advantage of being clear agars, enabling one better to observe the mycelium, trophocysts, and the initiation of sporangiophores. It is important to be certain that the pH of the agar remains above 7. McVickar's media, as stated by the author, was adjusted to pH 6.5, which is below the optimum for *Pilobolus kleinii;* this may explain the abnormal sporangiophores which McVickar described, and on which he based 3 new species of *Pilobolus*.

DEMONSTRATION OF A NEW GROWTH FACTOR AND ITS ASSAY

Once a pure culture of the fungus had been established, it was tested on a defined basal medium. The test medium was prepared by adding 10 ml. of sterile 3 per cent Difco Bacto agar solution to 10 ml. of sterile basal medium (TABLE I) in a Petri plate. Since spore inoculum was not feasible, blocks of dung agar removed from the margin of a growing *Pilobolus* colony were cut out and used as inoculum.

When this was done, no growth occurred. When Petri dishes with this basal medium plus dung decoction were tried, growth resulted. The same results were obtained when the synthetic agar for *Mucors* with thiamine added was tried (**6**).

The growth factor for *Pilobolus* has been named Coprogen. It has been isolated as a pure crystalline compound hitherto undescribed, the details of which will be reported at a later date.

TABLE I

COMPOSITION OF DOUBLE STRENGTH BASAL MEDIUM (10)

HCl-hydrolyzed casein	2.5 ml*	Folic acid	12 μg.
Sodium acetate	3.0 g.	Biotin	1.2 μg.
		B_{12}	2.4 μg.
L cystine	50.0 mg.		
DL tryptophane	100.0 mg.	Speakman Salts solution A	2.5 ml.(10)
Adenine	5.0 mg.	Speakman Salts solution B	2.5 ml.(10)
Guanine	5.0 mg.	Distilled water to pH adjusted to 7.6	250 ml.
Uracil	5.0 mg.		
Xanthine	5.0 mg.		
Thymine	5.0 mg.		
Thiamine	500 μg.		
Pyridoxine	100 μg.		
Ca pantothenate	100 μg.		
Riboflavin	200 μg.		
Nicotinic acid	500 μg.		
Choline	500 μg.		
Inositol	200 μg.		
PABA	200 μg.		

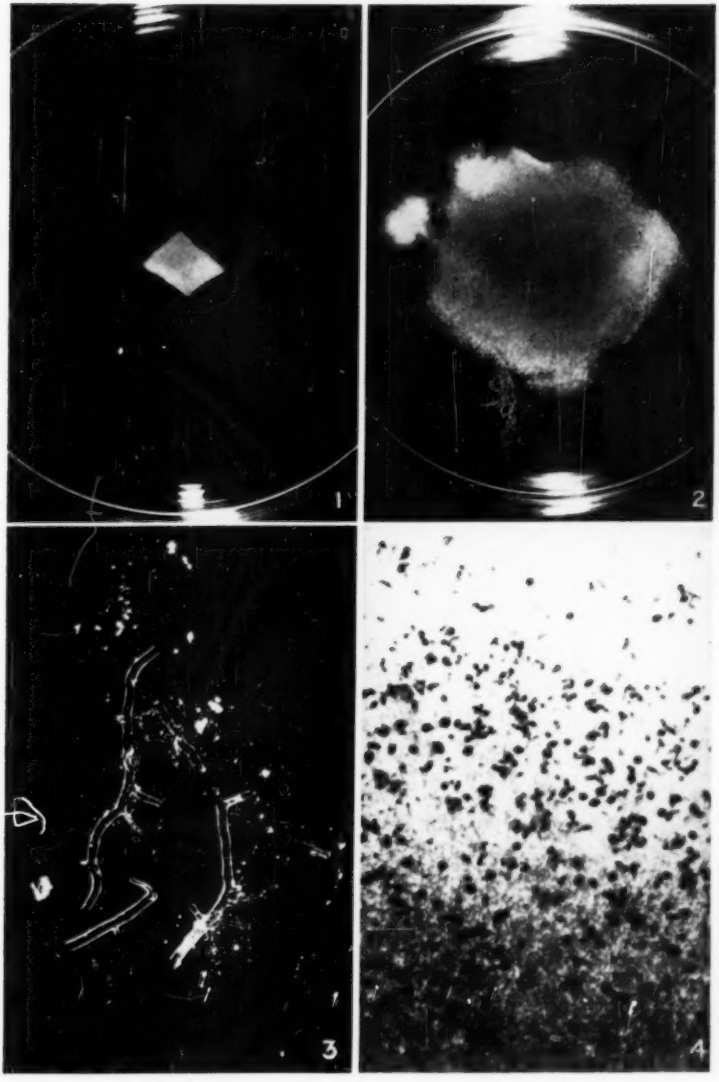
When used for liquid assay, 10 ml. of medium were added to 250 ml. flask. The test solution was added and volume adjusted to 20 ml.

* 1 ml. ~ 100 mg. of casein.

GROWTH IN LIQUID MEDIA

In an attempt to grow *Pilobolus* in liquid media, 25 ml. of dung decoction was sterilized in 500 ml. Erlenmeyer flasks. These flasks were inoculated with small blocks of dung agar from Petri plates containing the mycelium of *Pilobolus kleinii*, which immediately settled beneath the surface of the liquid. In 3 to 4 days a dense felt of mycelium was formed which, unlike most fungi, remained just below the surface of the liquid. From this felt of mycelium an abundant crop of sporangiophores was produced principally on a single day, although some were formed on several successive days. In many cases the sporangia were yellow instead of the usual black color, but later liquid cultures have indicated that normal sporangial production occurred. The sporangia were forcibly discharged just as they were on dung. As far as we are aware, this is the first time *Pilobolus* has been grown in liquid media. The mats were solid and could be readily lifted intact from the flasks.

Since the stock dung agar medium could be autoclaved, it was thought that the liquid assay of the factor in dung could be accomplished by autoclaving the basal medium with the fraction under study. Accordingly Erlenmeyer flasks were prepared with 10 ml. of the medium shown in TABLE I. Sterilization was accomplished



FIGS. 1–4. 1. Block of dung agar containing mycelium of *Pilobolus* used to inoculate the liquid assay. 2. The mycelial pad from an assay flask in which Coprogen was present. 3. Fragments of mycelium of *Pilobolus kleinii* after treatment with a Waring blender. 4. Portion of the mycelial pad somewhat magnified from FIG. 2.

by autoclaving for 15 minutes at 15 pounds pressure. After cooling, Seitz-filtered dung decoction and sterile distilled water were added to bring the volume to 20 ml., and the flasks were inoculated with blocks of agar (FIG. 1) containing some of the *Pilobolus* mycelium from a 3-day-old stock culture. These were then incubated at 28° C. for six days. The mycelium (FIGS. 2, 4) was lifted from the medium, the excess liquid was expressed, and the pad was dried at 110° C. for 16 hours. The results in TABLE II indicate a quantitative response.

TABLE II

ASSAY OF COPROGEN IN DUNG EXTRACT

Quantities of media in ml.			Dry weights of mycelium in mg. at 6 days
Dung decoction	Distilled water	Basal medium	
1	9	10	1.4
2	8	10	3.1
3	7	10	7.2
4	6	10	6.1
5	5	10	18.9
6	4	10	26.1
7	3	10	25.8
8	2	10	29.8
9	1	10	36.0
10	0	10	36.7

FACTORS INFLUENCING GROWTH OF PILOBOLUS

Stock dung agar plates were inoculated with blocks of agar containing *Pilobolus* mycelium and were incubated at four temperatures. There was essentially no growth at 36° C. except for a few filaments not more than 2 to 3 mm. in length; at 32° C. in the same period of time the plates were covered with mycelium but no trophocysts or sporangia were seen. In the same period at 28° C. growth covered the entire plates, and large numbers of trophocysts and sporangiophores were produced. At 24° C. growth was slower, but trophocysts were abundantly formed though fruiting had not yet been initiated at the time the plates were read.

An experiment was also set up to determine the pH range of growth for the *Pilobolus kleinii* isolate. Ten ml. of distilled water containing varying quantities of NaOH or HCl was sterilized in 250 ml. Erlenmeyer flasks, and then 10 ml. of Seitz-filtered dung

decoction was added. Each pH level was made up in sets of 4. One flask of each series was sacrificed before inoculating to determine its pH. The remaining three flasks were inoculated with small blocks of dung agar containing mycelium, and incubated at room temperature for 4 days. It will be noted that growth occurred on the alkaline side even at pH 10 but that the *Pilobolus* tended to bring the pH down to approximately 8–8.5. An acid pH of 6 or below completely prevented growth.

TABLE III

EFFECT OF PH ON GROWTH OF *PILOBOLUS KLEINII*

Initial pH	Final pH			Weight of mycelium in mg.		
	1	2	3	1	2	3
Uninoculated control 6.58						
3.2	3.3	3.3	3.2	*	*	*
4.1	3.9	3.8	3.9	*	*	*
5.3	5.1	5.2	5.2	*	*	*
6.2	6.1	6.4	7.3	*	*	4.1
7.2	7.9	8.0	8.0	5.3	4.7	6.4
8.0	8.1	8.2	8.1	4.7	4.7	4.9
8.9	8.2	8.3	8.3	3.4	3.5	4.3
10.1	8.6	8.6	8.6	4.3	3.9	4.3

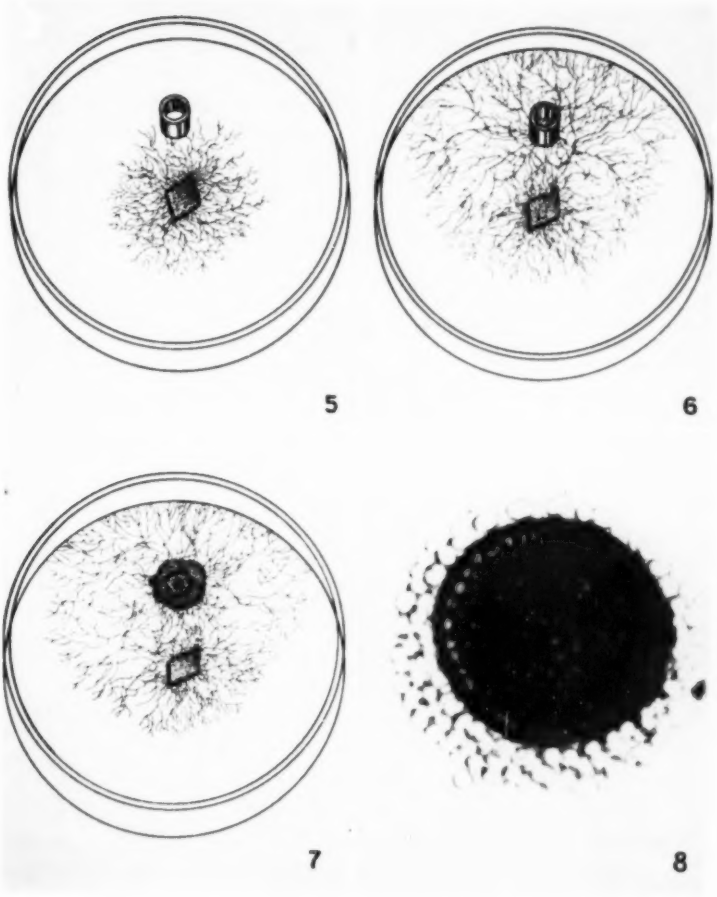
* Indicates no growth.

In any liquid assay it is desirable to use spores as inoculum (FIG. 8). With *Pilobolus* this was immediately found to be difficult because the spores are shot away from the surface of the medium against the sides and top of the container; they are contained in a gelatinous matrix which makes it difficult to separate them into a uniform spore inoculum; the conditions for consistent spore germination are unknown. A number of attempts were made to obtain spore germination. Ordinarily the spores from discharged sporangia fail to germinate when placed in tap water (neutral or acidified) or in dung decoction. Pretreatment in tap water of approximately 0.005 N HCl did not enhance germination. Spores suspended in tap water or acidified tap water for 24 hours at 37° C. showed no growth. Believing that perhaps a factor in the growing mycelium might have an effect, Petri dish lids which had many sporangia on them were flooded with the broth from cultures of actively growing *Pilobolus*. When these spores were examined, no

germ tubes were demonstrated. On several occasions, when suspended in alkaline dung decoction, alkaline trypsin solutions, or pancreatin dung decoction solutions, nearly 100 per cent germination resulted. However, even under these conditions a high percent of germination could not be predicted. Bacteria present in the enzyme solutions did not rule out their possible action. The age of the spores also seemed to be important. Because of the erratic nature of spore germination, this method of inoculation was abandoned, and the agar blocks were used in its place. Mycelial pads treated with a Waring blender did not result in a satisfactory inoculum because coenocytic Phycomycetes, unlike the other classes of fungi, possess no cross-walls or at most only a few; consequently, the breaking of the mycelium results in the cytoplasm flowing out into the medium with the resultant death of the fragment (FIG. 3).

PLATE ASSAY OF COPROGEN

Although the dry weight assay gives quantitative results, it seemed desirable to develop a more rapid and easier means of assay. Accordingly, the following procedure was found to give both rapid and reliable readings. Agar plates were prepared with the basal medium as previously described for the demonstration of the factor. Each plate was inoculated with a block of fresh stock dung agar containing actively growing mycelium of *Pilobolus*. The plates were incubated for 3–4 days at which time Coprogen was depleted from the block, and the *Pilobolus* mycelium had grown out for a distance of 1.5 to 3 cm. The edges of the colony were ragged and not too clearly defined because the main fungus filaments often grow farther than some of the shorter branched feeder filaments. The material to be tested was placed either in a paper disc or preferably in a cylinder on the agar at the edge of the colony. A typical assay is shown in FIG. 5. FIG. 6 shows a strong positive response. Where none of the factor was present, no further growth occurred. Readings can be made at 24 hours, but the final reading should be made at 48 or even 72 hours in order to check borderline cases. Certain conditions must be followed to make the assay reliable.



FIGS. 5–8. 5. Schematic diagram of the plate assay at the time the unknown solution is added to the cylinder. 6. A positive response to Coprogen of *Pilobolus kleinii* on an assay plate. 7. Positive response with a microorganism to Coprogen on the plate assay. 8. A sporangium of *Pilobolus kleinii* showing the sporangial wall and sporangiospores held within a gelatinous matrix.

1. The plates after inoculation must stand until the *Pilobolus* colony has reached its maximum growth diameter.

2. The pH of the basal medium must be above 7.

TABLE IV

ORGANISMS TESTED FOR THE PRODUCTION OF COPROGEN

Organism	No. of cultures tested	Response
Sarcina lutea	1	+
Spore-forming bacilli	25	−
Lactic bacteria freshly isolated from intestinal tract of a chicken	10	−
Red pigmented actinomycete	1	+
Pink yeast	1	−
Streptomyces A 54	1	−
Streptomyces A 105	1	+
Streptomyces A 120	1	+
Streptomyces A 37	1	−
Streptomyces A 88	1	−
Streptomyces A 307	1	−
Streptomyces A 81	1	+
Streptomyces A 247	1	+
Streptomyces A 153	1	−
Streptomyces aureofaciens A–377	1	−
Streptomyces rimosus	1	−
Saccharomyces cerviseae	1	−
Saccharomyces pyriformis	1	−
Saccharomyces fragilis	1	−
Ashbya gossypii	1	−
Candida pseudotropicalis	1	−
Unidentified yeasts	4	−
Torulopsis utilis	1	−
Proteus vulgaris	1	−
Alcaligenes bookeri	1	+
Escherichia coli	1	−
Staphylococcus aureus	1	−
Aspergillus carbonarius	1	+
Aspergillus nidulans	1	−
Penicillium sp.	1	+
Mortierella vinaca	1	−
Ustilago zeae	1	+

A + sign indicates the presence of Coprogen; a − sign indicates the absence of Coprogen by plate assay.

3. The cylinders containing the liquid to be assayed should always be placed with the edge of the cylinder just against the margin of the *Pilobolus* colony in order that a sample containing a small amount of the factor will show its activity.

4. Positive readings on questionable samples must not be made before the third day.

5. A positive reading can be made only when the fungus mycelium has grown under the cylinder and appears beyond it. In

strongly positive samples the mycelium will be heavy near the cup and grow to the side of the Petri dish.

6. No more than 3 samples should be placed on a plate.

With this type of assay it was possible to substitute for the assay cylinder a placement of a mold or bacterium to determine its activity (FIG. 7). By using such an assay a number of organisms were found which produced Coprogen. These are shown in TABLE IV.

NUTRITIONAL REQUIREMENTS OF THE GENUS PILOBOLUS

All of the species besides *P. kleinii* so far tested have shown an absolute requirement for this factor. These are summarized in TABLE V.

TABLE V

SPECIES OF *PILOBOLUS* TESTED AND FOUND TO HAVE AN ABSOLUTE REQUIREMENT OF COPROGEN

Species		Source
P. umbonatus (3 isolates)	deer dung	Tuxedo Park, New York
P. longipes	horse dung	Pearl River, New York
P. roridus (2 isolates)	horse dung	Pearl River, New York
P. kleinii	horse dung	Pearl River, New York

Since it had been the experience of one of us (**6**) that nearly all members of the order Mucorales would grow in a medium in which asparagine was the nitrogen source, a series of basal media were prepared in which asparagine was the principal nitrogous component. When these media containing vitamins and including Coprogen were tested, growth was as good as with the basal medium given in TABLE I. A further study of the omission of various vitamins indicated a definite B_1 requirement. Consequently, a simplified basal medium was devised which had the following composition: asparagine 2 gms., Speakman salts solutions A and B 2.5 ml. of each, glucose 10 gms., sodium acetate 6 gms., and 1000 ml. of distilled water.

Using this simplified formula, *Pilobolus kleinii* was carried through 10 successive transfers on this medium with the addition of highly purified preparations of Coprogen. The 10 serial transfers were carried through in agar plates using hyphal inoculum.

Further papers on the nutrition (**11**), methods of isolation, and properties of the pure Coprogen will appear elsewhere.

ACKNOWLEDGMENTS

The writers wish to thank Miss Marie Hauck for certain technical assistance. Thanks are also due to Mr. Arthur A. Jansson and Mr. Leslie McWilliam for preparing the illustrations.

SUMMARY

1. A factor necessary for the growth of *Pilobolus kleinii, P. longipes, P. umbonatus,* and *P. roridus* has been demonstrated.

2. This factor has been named Coprogen.

3. A stock medium consisting of dung decoction and oatmeal extract has been described for carrying species of *Pilobolus.*

4. A block inoculum assay has been developed for the assay of Coprogen in agar in Petri dishes.

5. The pH for maximum growth has been shown to be above 7.

6. Growth of *Pilobolus kleinii* in liquid media has been made possible resulting in a liquid assay that is both quantitative and qualitative.

7. Certain aspects of spore germination have been studied in the genus *Pilobolus.*

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A RELATION BETWEEN CELL MULTIPLICATION AND ALCOHOL TOLERANCE IN YEASTS [1]

JAMES R. TROYER

(WITH 4 FIGURES)

The cessation of many physiological activities of a microorganism despite the presence of an ample supply of nutrients and otherwise favorable environmental conditions has often been ascribed to "staling," a condition presumed to result from the inhibitory action of progressively accumulating products of metabolism. When the "staling" effect is manifested in industrial processes involving the biosynthesis of economically valuable substances, a knowledge of the mechanisms operative is obviously desirable. In the case of the commercial production of ethanol by yeast fermentation, the most significant metabolic product in this respect is that which is desired in the greatest quantities—the alcohol itself. The problem has been to develop methods of culture which afford increased yields by obviating the "staling" action.

Numerous investigators have from time to time measured the ethanol concentrations at which various fermentations cease, but the first systematic comparative survey of a number of yeasts was conducted by Gray (**1941**): from glucose utilization data obtained under reproducible conditions he defined alcohol tolerance as "the maximum percentage of alcohol (by weight) at which the percentage glucose utilization is no more than 1% below the percentage glucose utilization in the control flask of the same series." He was thus able to assign arbitrary alcohol tolerance values to each

[1] Paper No. 544 from the Dept. of Botany and Plant Pathology of the Ohio State University; based largely on a thesis submitted to the Graduate School of that institution in partial fulfillment of the requirements for the degree Master of Science. The generous counsel of Dr. W. D. Gray is gratefully acknowledged.

of 21 yeasts tested. He also described a lowering of these values at an elevated temperature and noted that measurement of carbon dioxide production in the presence of ethanol affords a convenient method of estimating the alcohol tolerance of a particular yeast. In other studies (**1946**, **1948**) Gray found a definite relationship between the amount of reserve material accumulated by a yeast and its alcohol tolerance: he reported that fat content especially varies inversely with alcohol tolerance.

The values derived by Gray, based as they are on rates of glucose utilization or carbon dioxide evolution, are empirical quantities of considerable practical utility. But their formulation indicates nothing concerning the manner in which ethanol is operative in effecting the observed reductions in glucose utilization. In Gray's experiments yeasts were cultured in a medium capable of supporting growth; since the measurements were made on a culture basis, the lowered glucose utilization rates might have resulted from effects of ethanol on (1) systems participating in carbohydrate breakdown, (2) cell multiplication (fewer cells might be operative), or (3) both sets of processes. Any attempt to elucidate the nature of the ethanol inhibition must first involve a consideration of these possibilities.

In this report an attempt is made to demonstrate that lowered population levels may largely account for reductions in glucose utilization per culture under the experimental conditions imposed by Gray.

MATERIALS AND METHODS

Six yeasts were employed; these are listed with the numbers under which they are cataloged in the stock culture collection of the Dept. of Botany and Plant Pathology of the Ohio State University:

1. *Saccharomyces cerevisiae* Hansen. D.C.L. Strain.
3. *S. cerevisiae* Hansen. R Strain.
20. *Hansenula anomala* (Hansen) Sydow.
22. *H. anomala* (Hansen) Sydow.
28. *S. cerevisiae* Hansen. Rasse M Strain.
31. *S. cerevisiae* Hansen. Brown-Forman Strain.

Five of these yeasts were studied by Gray (**1941**, **1946**, **1948**) and had been obtained from the collection of Joseph E. Seagram and Sons, Inc. Yeast *22* was secured from Dr. E. M. Mrak of the University of California under the designation *Hansenula anomala* var. *sphaerica* (Naegeli) Dekker; in conformity with the recent study of this genus by Wickerham (**1951**), however, the varietal name is not retained in this report.

A liquid glucose-phosphate-yeast extract medium (Gray, **1946**), denoted "standard," was used: 12.50 g. D-glucose, 6.25 g. KH_2PO_4, 8.75 g. Difco Yeast Extract, and distilled water to make 1 liter. Since inoculum, ethanol, and distilled water were later added to portions of this medium at the rate of four volumes of medium to one volume of additional liquid, the final concentrations in the culture vessels were 1.0 per cent D-glucose, 0.5 per cent KH_2PO_4, and 0.7 per cent yeast extract. The initial pH was adjusted to 4.4–4.6 (before autoclaving), measurements being made with a Beckman line-operated glass electrode.

For the preparation of inoculum a medium of similar type was used: 100.0 g. D-glucose, 5.0 g. KH_2PO_4, 7.0 g. Difco Yeast Extract, and distilled water to 1 liter. Yeasts from stock cultures carried in slant tubes (standard medium plus agar) were transferred to sterile inoculum medium contained in 40-ml. graduated centrifuge tubes; such cultures were incubated without shaking at 30° C for 24 hours. For inoculum designated "aerated" sterile 125-ml. portions of inoculum medium in 400-ml. bottles were seeded with yeast; air was passed from a compressed air line through sterile cotton and small-bore glass tubing to the bottoms of the culture bottles. Aerated yeasts were incubated 24 hours at room temperature (28–31° C).

To effect inoculation, cells were centrifuged out of inoculum cultures, washed with sterile distilled water, and resuspended in sterile M/15 KH_2PO_4 solution to the desired concentration. Proper amounts of suspension were added to culture vessels through sterile graduated pipettes.

Some fermentations were run in 50-ml. Erlenmeyer flasks, each containing a final volume of 25 ml. of liquid: 20 ml. of standard medium, 0.5 ml. of inoculum suspension, and 4.5 ml. of ethanol and

distilled water in varying proportions. Other fermentations were conducted in one-ounce soft-glass French square bottles, each with a final volume of 10 ml.: 8 ml. of standard medium, 0.2 ml. of inoculum suspension, and 1.8 ml. of ethanol and distilled water. Medium and water were placed first in the flasks or bottles, which were stoppered with cotton and autoclaved 20 minutes at 15 pounds gauge pressure; the ethanol was then added aseptically.

In all experiments except that concerned with the effect of temperature on cell numbers the cultures were maintained without shaking at 30° C; in the latter instance one series was held at 35° C, also without shaking.

Estimations of cell numbers were made turbidimetrically or by direct count. In the second case a Spencer Bright-Line Haemacytometer was used, and all well-defined buds which were more than one-fourth the size of the parent cell were counted. In the turbidimetric measurements cultures were shaken with glass beads to insure uniform suspensions, then allowed to stand one minute for dissipation of undissolved gases. Optical densities were read in a Klett-Summerson photoelectric colorimeter with a No. 42 blue filter against a blank of uninoculated medium; cell concentrations were calculated through the use of experimentally determined conversion factors.

For Yeasts 1, 3, 22, and 31 such turbidity measurements agreed rather well with direct counts; hence, these yeasts were employed in experiments in which populations were measured in this manner. As has been reported previously, attempts to correlate such measurements using Yeast 20 were fruitless because of great variability in size and shape of the cells (Kuehner, **1951**). In the present study, however, variations in results obtained with the closely related Yeast 22, while near the general limitations of the method, were not of sufficient magnitude to preclude use of this strain, provided care was exercised.

Glucose concentrations were estimated by the method of Stiles, Peterson, and Fred (**1926**).

EXPERIMENTAL RESULTS

Numbers of cells formed in ethanol. In these experiments Yeasts 1, 3, 20, 22, 28, and 31 were employed. For each yeast two

replicate series of fourteen 50-ml. flasks containing various initial amounts of ethanol were prepared. Twenty-four hours after inoculation haemacytometer counts were made, two per flask, and the values obtained for duplicate flasks averaged. These results are presented in TABLE I: the figures represent the average numbers of cells formed (final cells minus initial cells) as millions per milliliter for each alcohol concentration. In order that results for

TABLE I

AVERAGE NUMBERS OF CELLS FORMED (FINAL MINUS INITIAL) IN 24 HOURS BY SIX YEASTS IN CULTURES CONTAINING ETHANOL. YEASTS ARE ARRANGED FROM LEFT TO RIGHT IN ORDER OF INCREASING ALCOHOL TOLERANCE. (INITIAL CELLS: 1×10^6 PER ML.)

Initial EtOH	Yeast					
	20	22	1	28	3	31
mg./ml.	millions of cells per ml.					
0.0	62	71	31	60	38	59
29.4	41	49	32	57	36	62
36.8	23	44	24	65	41	58
44.1	6	33	22	52	33	57
51.5	0	26	15	50	30	52
58.8	0	3	8	27	26	33
66.2	0	1	2	1	3	3
73.6	0	1	1	0	0	3
81.0	0	0	0	0	0	0
88.3	0	1	0	0	0	0
95.7	0	0	0	1	0	0
103.0	0	0	0	0	0	0
117.7	—	0	0	0	—	0
132.4	—	1	0	0	—	1

the six yeasts may be compared, these averages are presented graphically in FIG. 1 as percentages of the number of cells obtained in the control flasks to which no alcohol was added.

In each case the number of cells formed in 24 hours decreased as the ethanol concentration increased, although not linearly. The reduction in population at a given alcohol concentration was much greater with some yeasts than with others. When the five (disregarding Yeast *22*) which were studied by Gray (**1941**) are arranged on the basis of the ethanol concentrations at which equivalent reductions in the numbers of cells formed occur, the order is $20 < 1 < 28 < 3 < 31$. Such ranking may be seen easily from

FIG. 1. This is exactly the order which was reported by Gray, as based on the ethanol concentrations at which equivalent reductions in glucose utilization were observed—that is, as based on alcohol tolerance as defined by him.

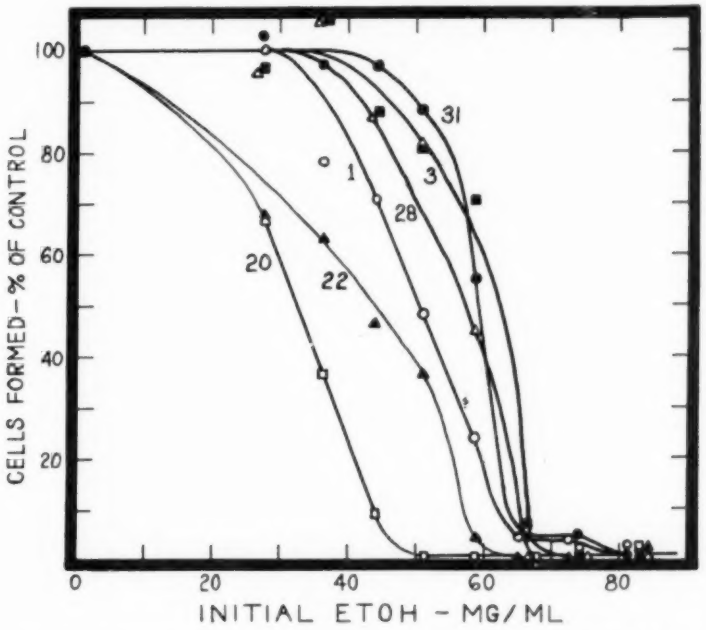

FIG. 1. Average numbers of cells formed (final minus initial) in 24 hours by six yeasts in cultures containing initial ethanol. Cell numbers are expressed as percentages of the results for the control flasks to which no ethanol was added.

Yeast *22*, which was not employed by Gray (his Yeast *22* was a strain of *Saccharomyces cerevisiae* Hansen), falls in the above series between Yeast *20* and Yeast 1; with this strain of *Hansenula anomala* (Hansen) Sydow the reductions in 24-hour populations in the presence of ethanol were large, but not as great as those found for the related Yeast *20*.

Effect of temperature. Since Gray (**1941**) reported that Yeast 1 exhibited a lower alcohol tolerance at 35° C than at 30° C, an experiment was conducted to determine the effect of temperature on

the numbers of cells produced in 24 hours in cultures containing ethanol. Yeasts 1, 3, 22, and 31 were employed. For each yeast four replicate series of six French square bottles containing various amounts of ethyl alcohol were prepared as previously described. Two series for each yeast were held at 30° C, and two at 35° C. Twenty-four hours after inoculation the cell populations were estimated turbidimetrically and the values obtained for replicate cultures averaged.

TABLE II

EFFECT OF TEMPERATURE ON AVERAGE NUMBERS OF CELLS FORMED (FINAL MINUS INITIAL) IN 24 HOURS BY FOUR YEASTS IN CULTURES CONTAINING ETHANOL. (INITIAL CELLS: 1×10^6 PER ML.)

Yeast	Temperature	Initial EtOH, mg./ml.					
		0.0	29.4	44.1	58.8	73.6	88.3
	°C	millions of cells per ml.					
1	30	27	26	22	16	4	2
	35	25	14	9	5	5	5
3	30	31	31	28	22	6	4
	35	21	14	9	7	6	6
22	30	69	52	33	7	10	6
	35	14	8	9	10	12	10
31	30	62	62	57	49	16	5
	35	18	11	9	9	11	9

From the results presented in TABLE II it is apparent that in each case fewer cells were formed at the higher temperature than at the lower; the exceptions in cultures containing 88.3 mg. ethanol per ml. do not seem significant since at both temperatures relatively few cells were formed. It should be noted that even in the control cultures to which no ethanol was added considerably fewer cells were formed at 35° C than at 30° C. (Results for Yeast 1 are also shown in FIG. 2.)

Aerated and nonaerated inocula. Gray (**1948**) reported that cultures of yeast from aerated inoculum exhibited lower alcohol tolerances than cultures prepared from nonaerated inoculum. Accordingly, an experiment was conducted to determine whether differences in population levels occur under these conditions. For

each yeast used (1, 3, 22, and 31) four replicate series of six French square bottles with various initial amounts of ethanol were prepared. Inoculum was cultured for 24 hours with and without aeration. Approximately equal numbers of cells from each type of culture were used in inoculating the test bottles: for each yeast

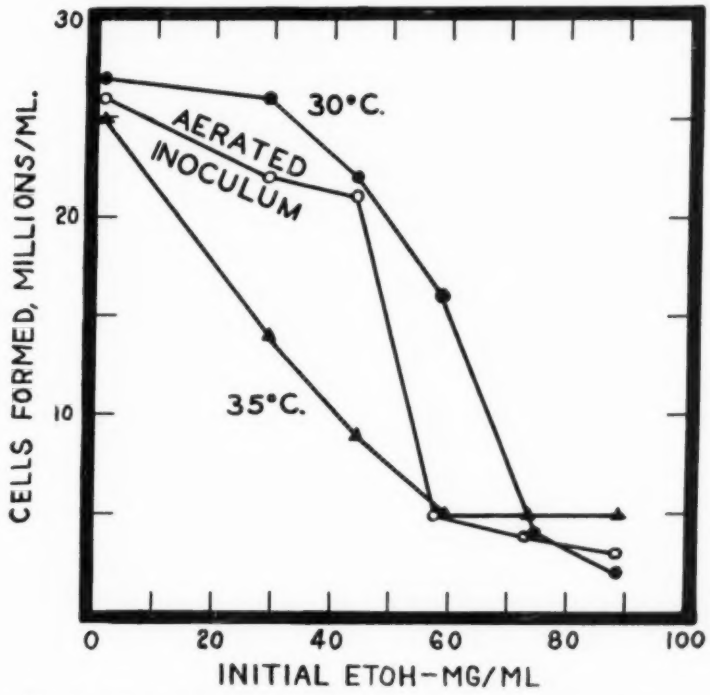

FIG. 2. Effect of temperature and aeration of inoculum on average numbers of cells formed (final minus initial) in 24 hours by Yeast 1 in the presence of ethanol. Cultures from nonaerated inoculum were maintained at 30° and 35° C, cultures from aerated inoculum at 30° C.

two series were inoculated with cells from the aerated culture and two series with cells from the nonaerated culture. After incubation for 24 hours at 30° C cell numbers were measured turbidimetrically and the values for replicate cultures averaged.

Results of this experiment are presented in TABLE III (data for Yeast 1 are also shown in FIG. 2). At almost every ethanol con-

centration fewer cells were formed by each yeast in the cultures from aerated inoculum than in those from nonaerated inoculum. Again, even when no alcohol was added initially there were differences between the numbers of cells formed in the two types of cultures; on the whole, however, these were small.

TABLE III

EFFECT OF ETHANOL ON AVERAGE NUMBERS OF CELLS FORMED (FINAL MINUS INITIAL) IN 24 HOURS BY FOUR YEASTS IN CULTURES FROM AERATED AND NONAERATED INOCULA. (INITIAL CELLS: 1×10^6 PER ML.)

Yeast	Inoculum culture method	Initial EtOH, mg./ml.					
		0.0	29.4	44.1	58.8	73.6	88.3
		millions of cells per ml.					
1	Nonaerated	27	26	22	16	4	2
	Aerated	26	22	21	5	4	3
3	Nonaerated	31	31	28	22	6	4
	Aerated	30	27	26	21	4	2
22	Nonaerated	69	52	33	7	10	6
	Aerated	59	47	15	8	8	9
31	Nonaerated	62	62	57	49	16	5
	Aerated	62	61	56	40	5	3

Time-course of multiplication in ethanol. In view of the observed reductions in 24-hour population levels described above, an attempt was made to ascertain more specificially the effects of ethanol on yeast growth by measuring the multiplication of four yeasts (1, 3, 22, and 31) over a period of 96 hours. For every yeast five sets of French square bottles, each representing a different initial ethanol concentration, were prepared; the cultures were held at 30° C throughout the experimental period. At various times the cell population in one culture from each set was measured turbidimetrically; results are expressed as averages of duplicate determinations, between which close agreement was found. Data from this experiment are presented in TABLE IV; results for Yeast 1 are also summarized graphically in FIG. 3.

Although the yeasts employed in this study differ from bacteria in manner of cell division and the growth pattern of the yeasts does not follow exactly that of bacteria, it is believed that the differ-

ences are not so great as to preclude analysis in terms of concepts developed by the bacteriologists. Growth curves obtained by plotting the data of TABLE IV are of the familiar type; in particular, in the three series of cultures having the lowest ethanol concentrations an exponential period of multiplication was closely approximated

TABLE IV

GROWTH DURING A 96-HOUR PERIOD OF FOUR YEASTS IN CULTURES CONTAINING INITIAL ETHANOL

Yeast	Initial EtOH	Hours after inoculation									
		0	3	6	9	22	33	47	54	72	96
	mg./ml.	millions of cells per ml.									
1	0.0	1	1	7	16	26	27	31	32	31	32
	29.4	1	1	3	10	26	26	28	29	31	32
	58.8	1	1	2	2	18	17	21	22	22	23
	88.3	1	1	2	2	3	4	4	3	5	7
	117.7	1	2	2	2	2	3	3	3	3	4
3	0.0	1	3	9	19	29	30	35	36	38	39
	29.4	1	3	6	16	29	29	34	35	36	38
	58.8	1	2	4	4	23	25	30	32	32	35
	88.3	1	3	3	3	3	3	4	13	27	31
	117.7	1	3	4	3	3	4	5	5	5	5
22	0.0	1	3	7	11	48	—	87	106	123	125
	29.4	1	3	6	7	42	60	80	89	104	124
	58.8	1	3	3	2	5	9	23	25	40	39
	88.3	3	4	5	5	5	5	7	8	8	12
	117.7	3	4	4	4	4	4	6	4	7	6
31	0.0	2	3	9	25	58	58	67	68	71	70
	29.4	2	3	5	16	57	57	66	66	70	68
	58.8	2	3	4	5	28	49	55	57	63	64
	88.3	2	4	5	4	6	7	9	30	53	57
	117.7	2	4	4	4	6	6	7	6	8	10

by each yeast. Hence certain growth constants described by Monod (**1949**) have been calculated. These constants, values of which are presented in TABLE V, may be briefly described.

Total Growth,

$$G = x_{max} - x_0,$$

expresses the difference between the initial (x_0) and maximum (x_{max}) cell concentrations (here cells per unit volume are used, rather than the dry weight per unit volume preferred by Monod). In every case as the ethanol concentration increases, the value of G

decreases, although the latter is certainly not a linear function of alcohol concentration (TABLE V). Also, the degree to which G for a yeast is decreased at a given concentration bears a relationship

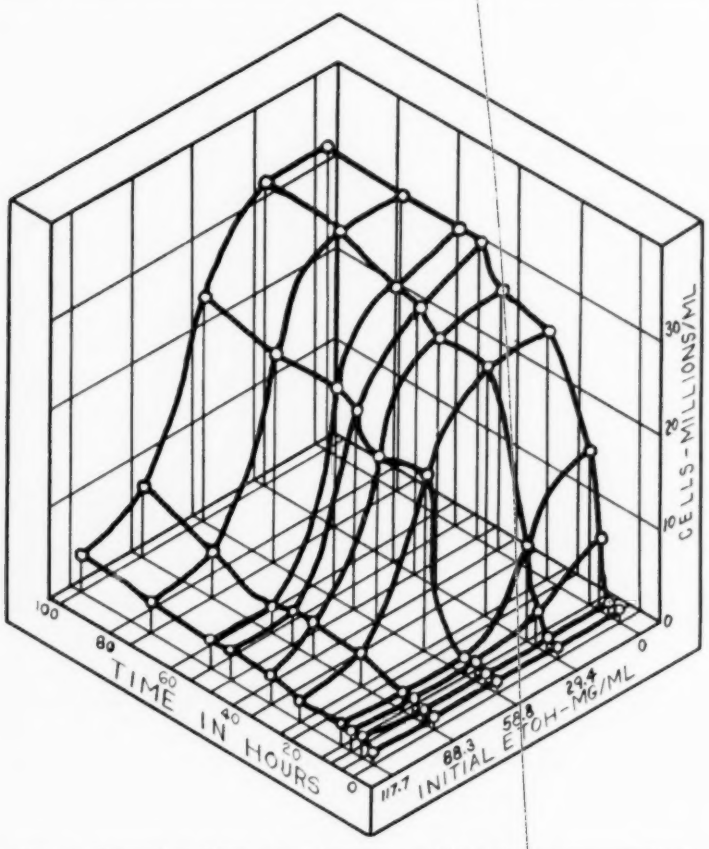

FIG. 3. Growth of Yeast 1 as a function of initial ethanol concentration and time.

to the alcohol tolerance of that yeast, as has been shown for 24-hour cultures in experiments described earlier.

Exponential Multiplication Rate is expressed by the equation

$$R = \frac{\log_2 x_2 - \log_2 x_1}{t_2 - t_1},$$

where $t_2 - t_1$ is any time interval within the exponential period of growth and x_1 and x_2 represent the numbers of cells at the beginning and end of that interval. In this study no exponential phase was observed for any yeast at ethanol concentrations of 88.3 and 117.7 mg. per ml., but R-values have been calculated for each yeast in the other three concentrations (TABLE V). From the results it appears that ethanol may have the effect of decreasing the exponential multiplication rate: only with Yeast 31 was this trend not followed exactly.

TABLE V

CALCULATED GROWTH CONSTANTS FOR 96-HOUR CULTURES OF FOUR YEASTS IN MEDIUM CONTAINING ETHANOL. (G = TOTAL GROWTH; R = EXPONENTIAL MULTIPLICATION RATE)

Yeast	Constant	Initial EtOH, mg./ml.				
		0.0	29.4	58.8	88.3	117.7
1	G	31	31	22	6	3
	R	0.77	0.52	0.43	—	—
3	G	38	37	34	30	4
	R	0.52	0.42	0.32	—	—
22	G	124	123	39	9	3
	R	0.43	0.42	0.10	—	—
31	G	69	68	62	55	8
	R	0.54	0.60	0.22	—	—

Two lag constants, the Lag Time (Lodge and Hinshelwood, 1943) and the Growth Lag (Monod) were also calculated; in some cases there was a regular increase in the values of these lag constants with increasing ethanol concentration. Among different yeasts the results varied considerably, however, and they are therefore not reported. From TABLE IV it is nevertheless obvious that an increase in lag was generally observed.

Evidently the growth of yeasts may be variously affected by ethanol in the medium. In this study the total number of cells formed varied inversely, although not proportionally, with the amount of alcohol present. This may have resulted largely through the reduction in multiplication rate during the exponential phase (and eventual elimination of this period), as well as from a possible

increase in lag. Other factors, such as the duration of the exponential phase, if there is one, and the rate of decrease in multiplication rate as growth tapers off are probably operative.

Cell numbers and glucose utilization. Reductions in yeast populations accompany the reductions in glucose utilization in the presence of ethanol reported by Gray for various experimental condi-

TABLE VI

AVERAGE NUMBERS OF CELLS FORMED (FINAL MINUS INITIAL) AND AMOUNTS OF GLUCOSE UTILIZED IN 24 HOURS BY SIX YEASTS IN CULTURES CONTAINING ETHANOL. (INITIAL CELLS: 1×10^6 PER ML.; INITIAL GLUCOSE: 10.150–10.460 MG./ML.)

Yeast	Initial EtOH	Cells formed	Cells formed, % of control	Glucose utilized	Glucose utilized, % of control
	mg./ml.	millions per ml.	%	mg./ml.	%
1	0.0	26	100.0	10.249	100.0
	29.4	21	80.8	10.460	102.1
	44.1	18	69.2	10.237	99.9
	58.8	7	26.9	1.127	10.9
	88.3	3	11.5	0.447	4.4
3	0.0	25	100.0	9.951	100.0
	29.4	25	100.0	9.959	100.0
	44.1	19	76.0	9.922	99.7
	58.8	6	24.0	5.765	57.9
	88.3	4	16.0	0.962	9.7
22	0.0	50	100.0	9.933	100.0
	29.4	19	38.0	4.777	48.1
	44.1	3	6.0	1.362	13.7
	58.8	3	6.0	1.087	10.9
	88.3	2	4.0	0.707	7.1
31	0.0	60	100.0	9.887	100.0
	29.4	57	95.0	9.892	100.0
	44.1	51	85.0	9.889	100.0
	58.8	8	13.3	5.891	59.6
	88.3	6	10.0	0.795	8.0

tions. The question which arises is whether the reduction in glucose utilization is a result of a lower population level, or whether the smaller population may be an effect of the utilization of less glucose.

An experiment was conducted in which both cell populations and glucose utilization were measured. For each yeast used (1, 3, 22, and 31) two replicate series of 50-ml. flasks were set up with increasing amounts of ethanol in successive flasks. The cultures

were held without shaking at 30° C for 24 hours; cell concentrations were then measured turbidimetrically and residual glucose determined. For Yeast 1 two additional series were prepared and similar measurements made after 48 hours. Values of duplicate cultures were averaged; results of the 24-hour experiments are

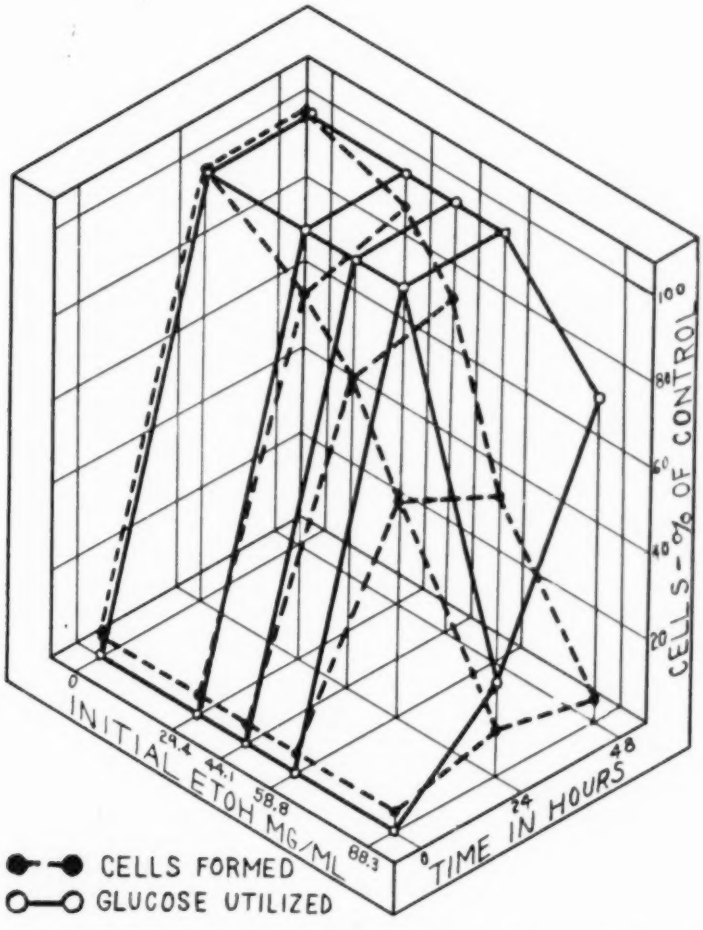

FIG. 4. Cell formation and glucose utilization in 48 hours by Yeast 1 in cultures containing initial ethanol. Numbers of cells formed and amounts of glucose utilized are expressed as percentages of the 48-hour values for control flasks to which no ethanol was added.

presented in TABLE VI, and the complete data for Yeast 1 are summarized in FIG. 4.

With these yeasts, cell populations fall off more rapidly with increasing ethanol concentration than does glucose utilization. If total growth depended only on the amount of glucose utilized, then the cells-ethanol and glucose-ethanol curves should decline sharply at nearly the same point. This, however, is not the case. Apparently reductions in cell populations in medium containing ethanol are not solely the result of decreased glucose utilization; the processes involved in cell multiplication seem to be affected at lower alchohol concentrations than are those connected with the disappearance of glucose.

DISCUSSION

The above experiments were designed to follow as closely as possible the procedures employed by Gray in his studies. From the results it is apparent that parallel reductions in cell populations accompany the reductions in glucose disappearance which he observed.

The number of cells formed by the six yeasts in graded series of cultures containing ethanol yield curves similar in shape to those reported by Gray for glucose utilization by five of the same yeasts under like conditions. Likewise, a decrease in alcohol tolerance with a rise in temperature is paralleled by reduced cell multiplication. Finally, the fact that cells from aerated and non-aerated cultures of the same yeast exhibit differences in alcohol tolerance is matched by the fact that such cultures also show differences in population levels attained in the presence of ethanol.

That ethyl alcohol is responsible for reduction in total numbers of yeast cells formed has been reported frequently—*e.g.*, by A. J. Brown (**1905**), H. T. Brown (**1914**), Richards (**1928c**), and others.

The reduction in multiplication which accompanies lower alcohol tolerance at elevated temperatures is also not unexpected: Richards (**1928a**, **1928b**) reported that above 30° C the process of budding is critically affected and growth falls off rapidly. Thus, the apparently increasing effectiveness of ethanol at temperatures above

the optimum (28° C) may be explained partly by the lowered multiplication rates which occur solely as a result of the temperature rise, and perhaps partly by a shift in the equilibria of the critical reaction involving ethanol (Rahn, **1945**).

Since aerated cells accumulate more fat, Gray (**1948**) has proposed on the basis of observations of Lindegren (**1945**) that a more ready coalescence of fat globules in cells of high fat content may account for the lower alcohol tolerance of aerated cells than of nonaerated cells of a yeast strain. Lowered multiplication rates of aerated cells in ethanol might also result from such greater coalescence of fat globules.

While most workers measuring ethanol effects on yeast growth have been concerned with the total growth, H. T. Brown (**1914**) studied 100-hour growth curves and reported reductions in both total growth and multiplication rate with increasing alcohol concentration. This conclusion is borne out in the present study, since total growth declined and the value of the exponential growth rate constant generally decreased with increasing amounts of ethanol until the exponential period vanished altogether.

The question posed earlier—whether the reductions in glucose utilization noted by Gray results from the effects of ethanol on the glucose breakdown mechanism, or on cell multiplication processes, or on both systems—may be partially answered by the experimental results described here. Two facts point to the idea that growth reductions result in the utilization of less glucose per culture (rather than that a lowered rate of carbohydrate breakdown limits multiplication).

In the first place, while 24-hour cell population-ethanol curves (FIG. 1) are similar in shape to the 24-hour curves of glucose utilization-ethanol for the same yeasts (Gray, **1941**), it is noteworthy that in every case the rather abrupt change in slope characteristic of both sets of curves occurs at a lower alcohol concentration in the case of the population curves than with the glucose utilization curves. Recalculations of Gray's original data, to enable comparison, are contrasted in TABLE VII with those of the present study; with these yeasts the approximate concentrations at which the rapid drop in multiplication begins are definitely lower than those at which glucose utilization declines. Secondly,

when populations and sugar disappearance were measured in the same culture, the former were reduced more by ethanol than the latter. This situation is especially apparent from FIG. 3.

While these results are certainly not conclusive, they support the hypothesis that a limiting of cell multiplication by ethanol is initial and primary in the alcohol tolerance effect. Such a concept is in accord with the statement of Rahn and Barnes (**1933**)

TABLE VII

COMPARISON OF THE ETHANOL CONCENTRATIONS AT WHICH CHARACTERISTIC SHARP REDUCTIONS OCCUR IN GLUCOSE UTILIZATION PER CULTURE AND TOTAL NUMBERS OF CELL FORMED. (GLUCOSE UTILIZATION VALUES RECALCULATED FROM DATA OF GRAY, 1941)

Yeast	Point of sharp reduction	
	Glucose utilization	No. of cells
	mg. EtOH per ml.	
20	<37.1	<29.4
1	59.4	29.4
28	81.7	36.8
3	81.7	36.8
31	>81.7	44.1

that in yeast cells exposed to damaging agents loss of the ability to multiply preceded loss of fermentative power. It further harmonizes with the report of Brockmann and Stier (**1947a**) that in the D.C.L. Strain (Yeast 1) utilization of the glucose initially present in a culture may proceed at a fairly constant rate per culture despite concomitant changes in yeast population.

Concerning the manner in which the effects of ethanol on yeast multiplication may be in turn reflected in glucose utilization, recent research offers a clue. Experiments such as those of Brockmann and Stier (**1947b**) indicate that the enzyme systems of yeast cells usually operate at below-capacity rates. These workers hold with Meyerhof (**1944**) that if maximum glucose utilization rates are to be attained the processes of phosphorylation and dephosphorylation must be synchronized. Since about two high-energy phosphate bonds are produced for every molecule of glucose fermented, the rate of dissipation of such bonds must balance the rate of their generation (Lipmann, **1941**); otherwise the adenylic acid

system may be saturated to such a degree that it can no longer serve as a phosphate acceptor in fermentative dephosphorylations.

In general, synthesis (cell-multiplication and accumulation of reserve materials) and hydrolysis (processes involving phosphatases) are the processes by which high-energy phosphate bonds are dissipated (Potter, **1944**). The experimental data of Brockmann and Stier (**1947b**) indicate that in growing cultures of Yeast 1 accumulation of high-energy phosphate bonds is avoided through the synthetic processes incident to cell multiplication. The rate of glucose utilization (per cell) is always higher during periods of active multiplication.

Thus, while the specific mechanisms through which ethanol inhibits yeast growth are undoubtedly complex, such reactions in preventing multiplication probably result in the accumulation of high-energy phosphate bonds—the resultant combined reduction in glucose utilization per cell and per culture appearing as the phenomenon of alcohol tolerance.

SUMMARY

Studies on the multiplication of four strains of *Saccharomyces cerevisiae* Hansen and two strains of *Hansenula anomala* (Hansen) Sydow under various conditions revealed reductions in growth which parallel the lowered glucose utilization rates previously reported.

Population curves for cultures containing various amounts of ethanol resemble those for glucose utilization by the same yeasts under similar conditions. A decrease in alcohol tolerance with elevated temperature is accompanied by reduced multiplication. Cells of the same yeast from aerated and nonaerated inocula, which exhibit differences in alcohol, also attain different population levels in the presence of ethanol.

The primary effects of ethanol on yeast growth are reduction in the total number of cells formed and lowering of the exponential multiplication rate.

Results of experiments involving estimation of cell numbers and glucose utilization in the same cultures indicate that the first effect of added ethanol is probably a reduction in multiplication, with glucose utilization by the culture as a whole declining only later.

It is suggested that the decreased growth effected by ethanol results not only in fewer active cells, but also in the accumulation of high-energy phosphate bonds. These two effects, which result in a reduction in glucose utilization, could account for the occurrence of the phenomenon of alcohol tolerance.

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CULTURAL STUDIES OF DACRYMYCES ELLISII

THOMAS J. BULAT

(WITH 9 FIGURES)

In 1888, Brefeld (1) described several species of fungi belonging to the family Dacrymycetaceae in volume 7 of his Untersuchungen. He included in his description the results obtained from germinating spores of *Dacrymyces deliquescens* in water and liquid nutrient cultures. These cultures produced thin-walled hyphae upon which were borne masses of conidia. The conidia ranged from ellipsoid to obovoid and were borne on short conidiophores either singly or in groups of two or more. He reports the size of these conidia as 7–3 × 5–2 μ.

The report by Brefeld appears to be the only work done on the cultural characteristics of this group.

In May, 1951, two collections of *Dacrymyces Ellisii* were made in the same general locality in Johnson County, Iowa. These two collections were similar morphologically except for the size of their spores. One had narrow spores averaging 14.7 × 5.7 μ while the other had broad spores averaging 13.6 × 7.5 μ.

Cultures were made on malt agar from the spores of each of these collections, GWM *6425* (narrow-spored) and GWM *6426* (broad-spored). This resulted in the development of several types of colonies. At first three types of colonies were observed, all of which were bright orange in color. They were: (1) giant colonies, (2) small colonies and (3) gelatinous pustules. Later, small white colonies arose from cultures of *6426*.

The manner in which colonies arose from various areas of the substrate after single point inoculations were made suggested that the spores were being projected in some manner. To determine whether this was the case, covers were changed from petri dishes

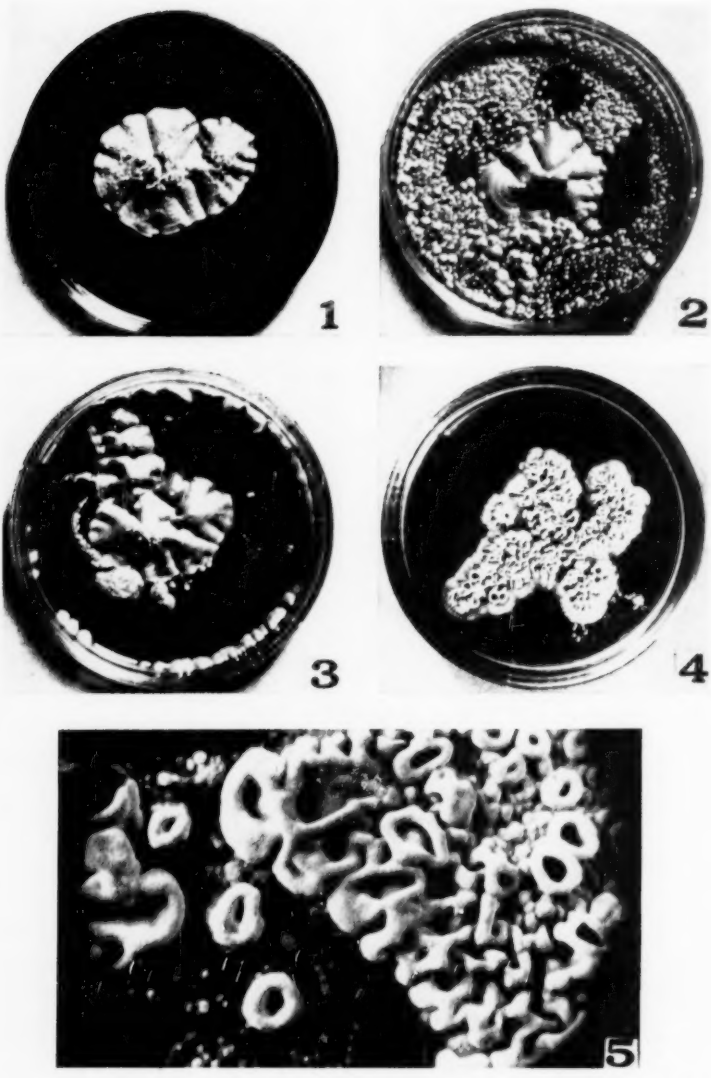
containing active colonies to petri dishes containing only sterile malt agar. This was done in hope that spores projected from the colonies had adhered to the cover and would in turn be projected onto the sterile agar thus starting new colonies. Within 3 to 5 weeks new colonies began to form on the agar indicating that the spores had been projected. In some of the more active cultures spores were projected to the covers of the petri dishes and caught in drops of water formed by condensation. Many of these subsequently germinated in the liquid.

DEVELOPMENT OF CULTURES

Single point inoculations were made in the center of petri dishes containing approximately 15 cc. of malt agar. In 5 days new growth could readily be seen. Mature colonies developed within 3 to 4 weeks.

In about one out of every ten inoculations only a single giant colony 2.5–4 cm. in diameter developed (FIG. 1). In the majority of inoculations, however, other colonies were also formed. These were small colonies similar to the giant colonies 0.1–1 cm. in diameter (FIG. 2, surrounding giant colony) and gelatinous pustules 0.1–0.5 mm. in diameter (FIG. 3, to right of giant colony). Often these colonies coalesced to form one large colony by confluence. The small colonies were sometimes so numerous that they coalesced before becoming fully developed and formed a single mat-like colony that covered the entire surface of the agar. The gelatinous pustules were at first discoid to pulvinate and then became irregular.

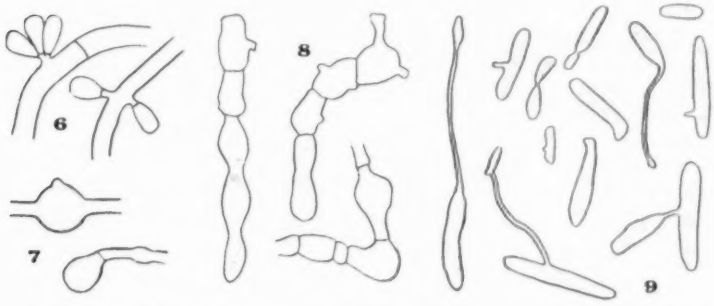
These colonies produced three types of spores. One type was ellipsoid to obovoid, 4–5 × 1.8–2.5 μ, borne singly or in clusters of 2 or more on short sporophores (FIG. 6). These spores gave evidence of being forcibly projected from their sporophores; however, no mechanism for this type of spore discharge could be ascertained. These are probably the same type of structures that Brefeld reported developing from spore cultures in liquid media. He did not observe the spores being projected because all his cultures were made in liquid. The second type consisted of thick-walled chlamydospores, spherical to obovoid, 6–12 × 5–8 μ, which may be intercalary or terminal (FIG. 7). The third type consisted



FIGS. 1–5. Cultural forms of *Dacrymyces Ellisii*. 1. Giant colony, × ½. 2. Giant colony surrounded by small colonies, × ½. 3. Giant colony with gelatinous pustules at right, × ½. 4. *Tilletiopsis* colony, × ½. 5. *Tilletiopsis* colony, × 6.

of the elongate ballistospores described below. The hyphae in older cultures often formed oidia (FIG. 8).

Approximately 300 transfers were made during an 8-month period. All were from cultures originally made from either *6425* or *6426*. The only discernible difference between *6425* and *6426* is that the latter is usually slightly lighter in color. All precautions were taken during the handling of these cultures to assure the absence of any contamination. During this period the colonies



FIGS. 6–9. Spore forms of *Dacrymyces Ellisii*. Drawn with the aid of camera lucida, × 1000. 6. Spores of *6425* which are forcibly projected. 7. Chlamydospores. 8. Hyphae breaking up to form oidia. 9. Pallistospores budding and germinating by repetition.

already described always developed. However, on four separate occasions a third type of colony forming ballistospores developed from *6426* (FIGS. 4, 5). These colonies are white when young and later become tan. They are at first almost completely composed of spores. More hyphae form as the culture ages. The spores are elongate and slightly curved, 11–25 × 2–3 μ, averaging 17× 2.6 μ (FIG. 9). These spores germinate by budding and by repetition, and they are forcibly projected. Oidia will form from hyphae in the older cultures.

DISCUSSION

The forms which appear in the various cultures of *Dacrymyces Ellisii* exhibit a little known phase in the life cycle of that fungus. The most interesting aspect of the results obtained in this work was the appearance of spores which were projected from their sporophores and the occurrence of certain spores which germinated by

budding and by repetition. The white colonies which arose from *6426* have all the characteristics of the genus *Tilletiopsis*. It is believed that they are definitely members of this group. Furthermore, the projected spores found in the other colonies also show a resemblance to the family Sporobolomycetaceae.

The position of the Sporobolomycetaceae has always been questioned. There have been various opinions as to its correct taxonomic position. Buller (**2**) thought it to represent reduced forms of Basidiomycetes. Derx (**3**, **4**) has also stated that the group probably belongs in the Basidiomycetes. In speaking of the genus *Tilletiopsis* Nyland (**6**, **7**) states that the lack of parasitism alone is hardly sufficient reason for excluding that genus from the Ustilaginales. Olive (**8**) has expressed the opinion that the Sporobolomycetaceae may be related to the Tremellales. Martin (**5**) has included the Sporobolomycetaceae as an addendum to the Tremellales in his recent Revision of the North Central Tremellales. Others have placed these fungi in the false-yeast group.

The forms described in this paper show clearly that such fungi may represent part of the life cycle of *Dacrymyces Ellisii*. The life cycle of this species, therefore, would consist of the perfect stage characterized by the formation of basidia, an imperfect stage marked by the formation of conidia and an intermediate phase which takes the form of ballistospores which germinate by repetition and budding.

This constitutes further evidence that the Sporobolomycetaceae have a definite taxonomic relation with the Tremellales.

Studies are being made at the present time of cultural forms of other genera of the Tremellales.

This work was done under the supervision of Professor G. W. Martin.

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SOME VIEWPOINTS ON THE PHYLOGENY OF RUST FUNGI. I. CONIFEROUS RUSTS

E. E. Leppik

(with 5 figures)

Most recent mycologists (Dietel, Faull, Gäumann, etc.) agree with A. Möller's (**17**) proposal to derive the Uredinales or rust fungi from the order Auriculariales of the class Basidiomycetes. Their point of view rests mainly upon the marked similarity which exists in the structure of the phragmobasidium and the formation of basidiospores of the Auriculariales and Uredinales. There are two further hypotheses, that of de Bary (**1**) which derives the Uredinales from the Ascomycetes, and that of Brefeld (**2**) which connects them with the Zygomycetes. These hypotheses have not found further support since the question was thoroughly discussed by Gäumann (**4**, pp. 421, 586–592). But mycologists do not agree too well when it comes to the classification of this group of fungi. More than 4000 recent species of rusts are represented in such a variety of sporulating forms that it is difficult to subordinate all of them to the definite groups.

Up to the present time no definite phylogenetic system of Uredinales could be established. Gäumann (**5**, p. 586; **6**, p. 303) characterizes the present situation as follows: There is a complete anarchy in regard to the significance of all forms and types of life cycles of these fungi. How have they arisen, and how are they related? The answer to this question is difficult, for the Uredinales belong to the oldest known fungi and they have survived to our time, isolated as *"living fossils."* They have not, however, left us *petrified fossils* of their ancestors. Thus the answer cannot be given without considering at the same time the phylogeny of the Uredinales, their systematic division and the origin of their biological specialization.

Recently the history of rust fungi has been studied in connection with the phylogenetic and hologenetic development of their host plants, which method provides a new approach to the question of their phylogeny and classification. The present article attempts to summarize some results which have been reported in Germany and are not readily accessible to American mycologists.

THE PRESENT CLASSIFICATION OF RUST FUNGI

The present taxonomy of the rust fungi is based on the morphological structure of their teliospores. This method of classification, however, has been applied by various authors in different ways, and cannot be accepted without criticism (Leppik, **10**).

Although the teliospores may provide perfect characteristics for generic identification, their distinctive value cannot be used with the same effect for the classification of families and suborders.

During the long process of evolution the rust fungi have several times eliminated their former teliospores and have developed entirely new forms of spores in some other stage of the life cycle which have taken over the function of teliospores. Therefore, the teliospores of different phylogenetic groups may be similar but not always genetically identical organs. In most cases they are formed from the binucleate urediniomycelium. In *Coleopuccinia* they are derived from the aeciomycelium, in *Endophyllum* directly from aeciospores, in *Uredinopsis* from the intrasoral mycelium, etc.

Still more frequent are the cases where the teliospores in genetically related groups, responding to different ecological environments, have changed their shapes to unrecognizable forms. In these cases the teliospores may be genetically identical but are not similar morphologically. Because of their extreme plasticity the phylogenetic value of teliospores cannot be very great, and the term "teliospore" can be applied in a functional rather than in a genetic meaning. The question of origin and historical development of teliospores can therefore be approached with hologenetic viewpoints rather than phylogenetic, and will be discussed in a later chapter.

On the other hand, the stability of forms of basidiospores, urediniospores and pycnospores does not by any means furnish a better

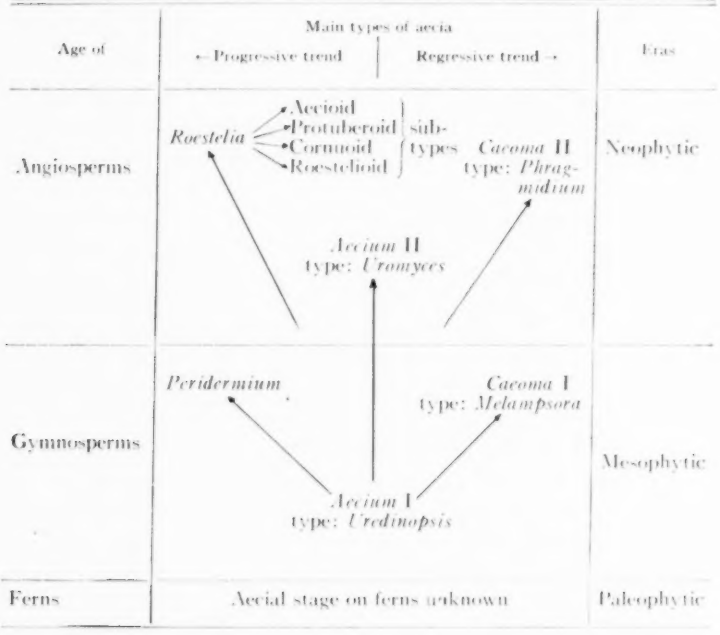
basis for the phylogenetic classification of rust fungi than the diversity of teliospores.

THE EVALUATION OF AECIA FOR PHYLOGENETIC PURPOSES

The single-celled, binucleate aeciospores are typical of all heteroecious rusts. Their morphologic structure is stable and the slight variations in their dimensions and cell-wall structure have no special significance for phylogenetic distinction.

TABLE I

PRINCIPAL TENDENCIES IN THE DEVELOPMENT OF AECIA



More valuable for phylogenetic purposes are the aecia or aecidia, the special sori where the aeciospores are formed. The principal tendencies in the development of aecia are sketched on TABLE I and can be compared with the main evolutionary trends of coniferous rusts, pictured on FIG. 1.

According to this table, the *aecium* I, type *Uredinopsis*, with well-developed pseudoperidium, could be one of the oldest in this group. It presumably arose as early as the end of the Paleozoic Era and reached stability in the Mesozoic on gymnosperms in a comparatively dry climate. This type delivered two early branches: *peridermium*, with an exceptionally thick pseudoperidium adapted to an especially dry environment, and *cacoma* I of *Melampsora*,

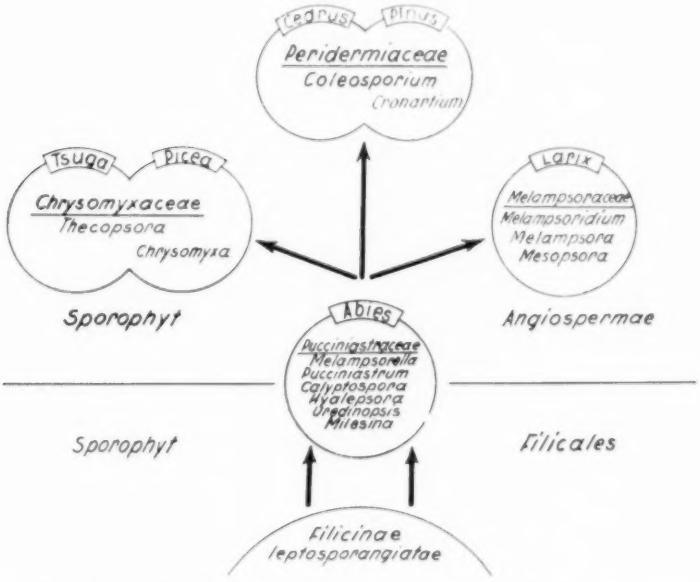


FIG. 1. Some main evolutionary trends of coniferous rusts on Abietaceae, as these can be established according to morphological structure and biological specialization of recent forms.

with a reduced pseudoperidium, adapted to a moist climate. Evidence is so far morphological, phylogenetic and Łologenetic (Leppik **8**, **10**, **14**).

Later this type, without much change in its morphological structure, reached angiosperms, where it was distributed as the *aecium* II of *Uromyces* in the *Pucciniaceae*, the largest group of rust fungi.

In some cases the *Uromyces*-type could not adjust itself to special climatic conditions and was therefore replaced by *roestelia* and *cacoma* II.

Special attention should be given to the *roestelia* type of *Gymnosporangium*. The aecia of this type possess a modern mechanism, which regulates the distribution of spores in connection with changing weather conditions. This mechanism enables it to hold back the spore material in the sorus until the combination of temperature, humidity and wind velocity has reached a most favorable index for distribution. When this point is reached, a special mechanism empties the sorus and closes it again as long as new spores are ripening (Leppik **1949**).

This new achievement of a young rust family has proved effective and the mechanism has already been modified in several special cases. In fact *roestelia* has split itself into several subtypes or "typomeres": aecioid, protuberoid, cornuoid and roestelioid, which are provided with clear morphological characteristics (see TABLE I and Leppik **14**, from manuscript).

In this polymorphism and variability of *roestelia* subtypes is reflected the phylogenetic youthfulness of the genus *Gymnosporangium*. This is a new trend within the higher rust fungi, a mechanism to regulate the distribution of their spore material in connection with weather conditions.

In this way the arrangement of aecial types according to their historical development may provide a framework for the further phylogenetic considerations of the rust fungi.

AECIA AS INDICATORS OF CLIMATIC CONDITIONS

The aecia of rust fungi are intended to protect immature aeciospores against drought and to facilitate the distribution of these spores after their maturation. The protection of these spores against low temperature may be of some importance in localities where frost in the early spring is common. Several constant aecial types are, therefore, characteristic of certain weather conditions and may be evaluated as indicators of the climate. Except in some special cases, the aecia reflect the weather conditions of spring and early summer, which is the season of their appearance. A historical development of aecia may consequently reveal the climatic change of the past (Leppik **9**, **12**, **14**, **15**).

The general picture of a climate, as it is mirrored by different types of aecia, may be completed according to other sporulating

forms of rust fungi. Protected urediniospores point to a dry summer, whereas the development of a protective covering by the teliospores suggests a cold winter. To be sure, these suggestions can have practical value only if the origin of different types of rust sori can be connected with the historical development of their bearers.

The present-day distribution of types is not always decisive. The rusts with well-protected sori are also able to live in a moist and warm climate, although the opposite weather conditions are the essential prerequisites for their original development.

In the case of coniferous rusts, the comparative phylogenetic and hologenetic studies of these rusts in connection with their host plants enables us to restore the origin and historical development of some main types of aecia (see later). These studies also allow us to make some conclusions concerning the climatic conditions of the past, which have been responsible for the development and accomplishment of certain definite types of aecia.

The ancestral *prototype* of the aecia of coniferous rusts presumably had a tubular or cup-shaped, thick-walled pseudoperidium, which provided sufficient protection for the immature aecidiospores against drought in a moderately dry climate. This prototype can be assumed according to the morphological structure of numerous living representatives of coniferous rusts. Among the recent types, the *aecium* I of Pucciniastraceae comes closest to the ancestral prototype of the aecium.

The geographical distribution of recent fir forests in the Northern Hemisphere, the residence of *aecium* I on *Abies*, enables us to realize the climatic environment in which the early development of the aecial prototype has taken place. The moderately dry climate of fir forests with well established seasons, differs sharply from that of the tropical rain forests, the place where the ancestors of coniferous rusts presumably lived near the end of the Paleozoic Era. The emigration of rust fungi from the moist paleotropic climate into the temperate coniferous forests of the Northern Hemisphere was evidently the main cause of the rearrangement of their sporulating organs. All other forms of spores except the basidiospores received peridial covering, whereas the teliospores were embedded in the host tissue. Since that time, Urdineae sorosporae

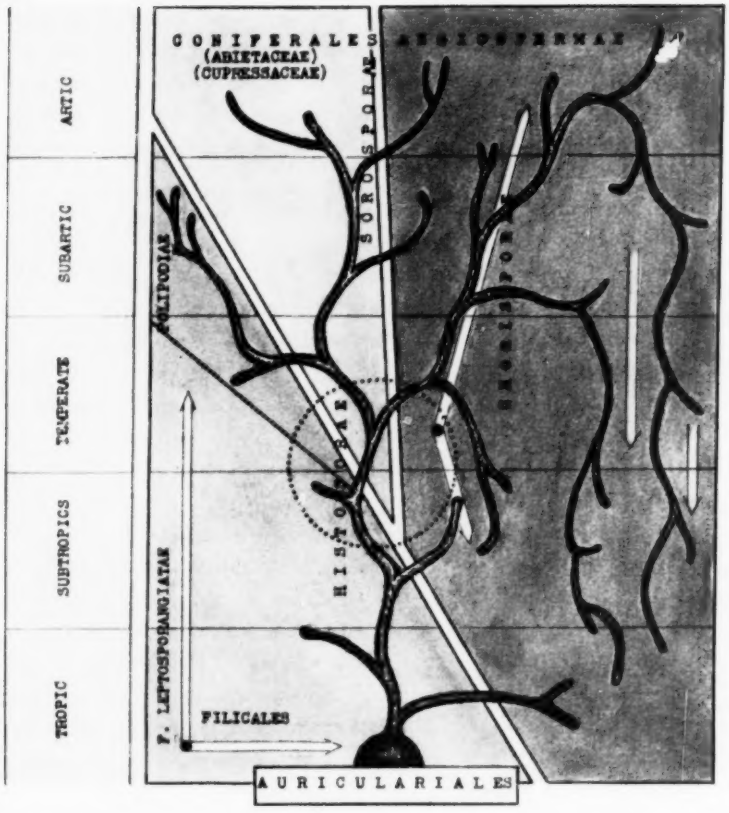


FIG. 2. An assumed genealogical tree of rust fungi, projected on the shaded fields of the host plants of these fungi. Originating from the tropical Auriculariales the main road of rust fungi leads from tropical ferns over conifers to the temperate and arctic angiosperms. White arrows indicate the main evolutionary trends of the host plants (see text).

became associated with the coniferous family Abietaceae and preserved this specialization to the present day: Their further evolution is closely connected with the historical development of Abietaceae. The splitting of the Abietaceae into the recent genera *Abies*, *Picea*, *Pinus*, *Larix* and *Cedrus*, which were adjusted to the various special climatic conditions, followed the splitting of the aecial prototype into the recent *aecium* I, *caeoma* I, and *peridermium*. All

these types represent the specific climatic condition in which they live.

In the morphological structure of *caeoma* I of *Melampsora* is reflected the damp climate of the temperate and subarctic forest regions of the northern hemisphere. A short, but moist spring with long days and short nights caused only a slight change of temperature between day and night. The moist soil covered with mosses, the retarded evaporation in the half-shadowed forests, and the moderate air currents are the further climatic prerequisites for the development of *caeoma* I. A flat caeoma without a pseudoperidium is able to produce more spores than an aecium, and is able to expose all of them to the air current at the same time. A tubular peridium would hinder this procedure. In the morphological structure of teliospores of *Melampsora* is further mirrored a long and hard winter of the northern hemisphere.

Larix, the main host of *caeoma* I, has responded to these special climatic conditions by changing its permanent needles to deciduous leaves, which is a rare phenomenon among the conifers. Other host plants of *Melampsora*, such as *Salix*, *Populus*, Orchidaceae, etc., that live in the same environment with *Larix* and *Melampsora*, and are exposed to the same ecological conditions, are all well-known mesophytes.

Peridermium, with several peridial layers, is one of the best protected aecia in the exceptionally dry climate of the pine forests. It can therefore thrive on *Ephedra* even in the half-desert areas of America and Asia.

THE RELATIONSHIPS BETWEEN THE DIFFERENT TYPES OF AECIA

There is much similarity in the morphological structure of aecia and aeciospores of coniferous rusts. The anatomy of the epispores, the formation of spores in chains, and the development of tubular pseudoperidia, all point to the common origin of *aecium* I, *caeoma* I and *peridermium*. They all inhabit closely related genera of the Abietaceae, which fact makes the mutual relationship of these rusts and the origin of their aecial sori from a common prototype still more conclusive.

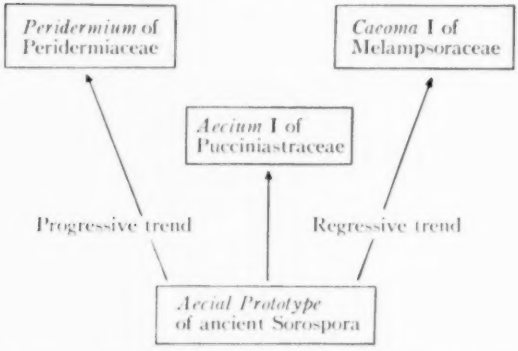
But none of the existing types can represent the aecial prototype, though *aecium* I of Pucciniastraceae is closer to it than any

other sorus. In all probability the present aecial types have evolved from a common prototype of ancestral Sorospora, as sketched in TABLE II.

There is an analogy in this scheme and in the evolutionary trends of coniferous rusts, as these are demonstrated on the recent groups

TABLE II

THE HISTORICAL DEVELOPMENT OF AECIUM I, CAEOMA I AND PERIDERMIUM FROM A COMMON PROTOTYPE



(see FIG. 3). The historical development of these main groups of Uredineae sorosporae also coincides with the evolution of the Abietaceae (see TABLE III).

Among the recent types, *aecium* I stays closest to the proto-aecium of Sorospora (see below). Concerning the development of the pseudoperidium, the *peridermium* is a progressive and *caeoma* I a regressive trend from this aecial prototype.

The *caeoma* II of *Phragmidium* evidently has no relationship with *caeoma* I and should be provided with another name.

The striking similarity in the morphological structure of the *aecium* I of *Uredinopsis* and the *aecium* II of *Uromyces* is very significant. Aeciospores of both groups have the same cell-wall structure, are formed in chains, and possess intercalary cells. Further evidence of the relationship between *aecium* I and *aecium* II can be obtained from hologenetic considerations.

The question of a genetic relationship between Pucciniastraceae (*aecium* I) and Pucciniaceae (*aecium* II) is one of the most fascinating problems, not only in the phylogeny of rust fungi, but

also in the origin of angiosperms, and should be discussed in another article.

The *roestelia* of *Eugymnosporangium* is a new sporulating form among the rust fungi. In spite of a marked difference in its morphological structure, this type has a clear phylogenetic and

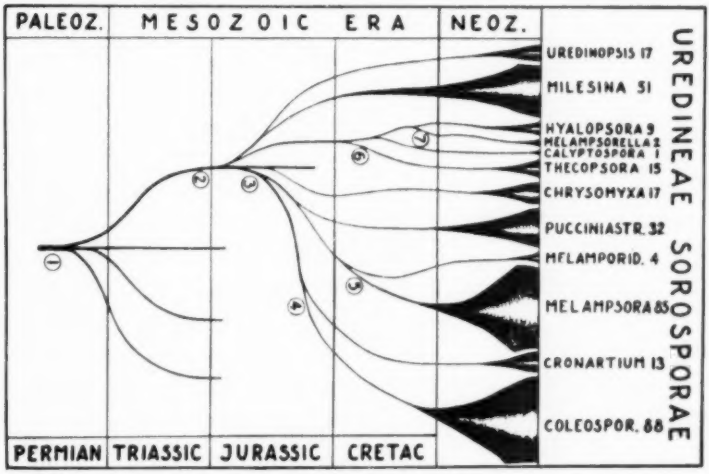


FIG. 3. Genealogic tree of Sorosporae. Encircled numbers are some points which may be connected with geological time table (see text).

hologenetic relationship with the aecium. Except some protuberoid forms, the main part of the subtypes of *roestelia* can be derived from the still living ancient cupulate sori of *Gymnotelium*.

RELATIONSHIPS AMONG THE DIFFERENT GROUPS OF CONIFEROUS RUSTS

The existence of a close relationship between different groups of coniferous rusts on the Abietaceae seems to be beyond doubt. The morphological structure of the aecia and aeciospores (see above), the coincidence of their biological specialization, and the geographical distribution of present forms makes the origin of these rusts from a common ancestral group highly probable. In spite of many controversies about the structure of their teliospores, most of the taxonomists at present are combining all coniferous rusts into a single group. But even the great variation of telio-

spores can now be interpreted as a result of several hologenetic tendencies within the same group.

In spite of a close relationship among the coniferous rusts none of the existing groups can represent the prototype for these rusts. All recent genera and families of these rusts have been, for many ages, parallel evolving groups, each without ancestral rights for the recent suborder of coniferous rusts.

The often used name "Melampsoraceae" for the coniferous rusts is rather confusing, and from the phylogenetic standpoint, quite untenable. The genus *Melampsora*, which is characterized by *caeoma* I, is a new, partly regressive trend, and cannot be considered as either a prototype or an ancestral group, for these rusts. Gäumann (**6**, p. 329) has already abandoned the old viewpoint and preserved the name "Melampsoraceae" only for a restricted group of coniferous rusts, the bearers of *caeoma* I. But every group of these rusts preserved some of the traits of their archaic ancestors. A comparative study of recent groups in connection with phylogenetic and hologenetic trends among these rusts and their hosts enables us to restore the morphological picture of their ancestors.

In a former article (Leppik **10**) it was suggested that all families of coniferous rusts that had a prevalent tendency to close their main forms of spores into definite sori, be united into a suborder Sorosporae.

There is some heuristic value and also some practical use in providing the ancestral group of Sorosporae, the bearer of the aecial prototype, with a provisional name *Sorospora*. Its existence in the late Mesophytic is beyond doubt.

In view of the recent rich flora of the suborder Sorosporae, it is not difficult to establish the main morphological characteristics of the ancient *Sorospora*. Most typical of *Sorospora* is its formation of spores in specially protected sori. Except basidiospores, all other spores are covered with a pseudoperidium and are embedded in host tissue. Teliospores are, in the beginning of this new evolutionary trend of rust fungi, disorganized in the host tissue, but have the clear tendency toward the well-organized subcuticular sori. Urediniosori are also covered with a pseudoperidium.

Amphiospores may be assumed for *Sorospora*, because they are typical for some lower coniferous rusts, but are gradually disappearing in higher forms.

In this image of combined sporulating forms of *Sorospora* is reflected a picture of an archaic climate from the late Mesophytic Era. A moderately dry spring and summer are the necessary prerequisites for the evolutionary development of a protective pseudoperidium of aecio- and urediniosori. A moderately cold winter is required for the genesis of the different types of teliospores which are represented in the suborder Sorosporae. The variation of the teliospores further mirrors several subtypes of the winter climate of the past.

THE PLACE OF ORIGIN OF CONIFEROUS RUSTS

According to the available material and the above presented conclusions, some assumptions can be made about the place of origin of the coniferous rusts.

All main soral types of suborder Sorosporae reflect in their structure a temperate or subarctic climate, which weather conditions may also be assumed for the development of ancestral *Sorospora*.

The aecio- and uredosori are covered by a well-developed pseudoperidium, which protects the maturing spores against drought during the spring and summer, i.e. the seasons of their appearance. The morphological structure of the teliospores and the position of the teliosori in the host tissue mirror the different accommodations that have occurred to protect these spores against low temperatures in winter. The origin and development of a protective pseudoperidium in a moist climate, or of winter spores in the tropics, would be quite improbable. Neither is it possible to imagine the origin of the deeply rooted heteroecism of coniferous rusts in a region without climatic seasons.

The historical development and recent geographical distribution of Abietaceae exclusively in the Northern Hemisphere restricts the origin and living area of the coniferous rusts to this territory of their host plants.

Under these conditions it is rather discouraging to look for the place of origin of coniferous rusts in recent temperate Europe or

America, which provinces had a tropical climate up to the middle of the Cenozoic Era. *Sorospora* definitely could not arise in the paleotropic Europe or America, where the weather conditions have been quite unsuitable for this fungus until the late Tertiary Period. According to the paleontological records occasional climatic prerequisites existed in the recent arctic regions during the Jurassic and Cretaceous Periods.

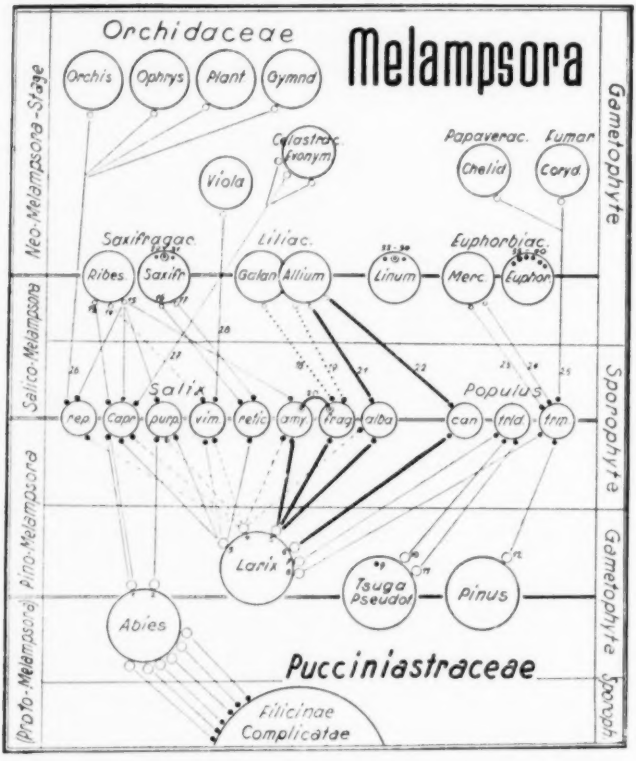


FIG. 4. The hologenetic ladder of biological specialization of *Melampsora*. According to this ladder the phylogenetically oldest species of the *Proto-Melampsora* stage form the aecia on *Abies* and teliospores on ferns. The next stage of *Pino-Melampsora* connects the Abietaceae with the Salicales. Further, the youngest species of the *Neo-Melampsora* stage step from the Salicales to the higher angiosperms. (From Leppik **12**, 1938. For explanations see text.)

It is quite certain that the birthplace of ancestral *Sorospora* was in the fir-fern forests of *Proto-Abies* in the Northern Hemisphere. Both host plants, *Proto-Abies* and a young fern group of *Polypodiaceae*, adjusted themselves to live in the northern temperate provinces where they formed extensive fir forests, with ferns as the main undergrowth. This is clearly demonstrated in the biological specialization of Pucciniastraceae (see above). Other conifers of the Taxodiaceae and Araucariaceae could not live in the territory of fir forests. The invasion of coniferous trees toward the north was continued by the formation of spruce and hemlock forests, the habitat of *Chrysomyxa*.

Accompanied by *Melampsora*, the genus *Larix* could force its way still farther north to the moist subarctic regions. In the hologenetic ladder of *Melampsora* is mirrored the historical development of the tamarack forests. Into the mixed *Larix-Salix-Populus* forest enter gradually a number of angiosperms: *Saxifraga, Ribes, Allium, Calanthus, Evonymus, Viola*, different Orchidaceae, etc. (see FIG. 4).

After the cooling of the northern hemisphere during the Cenozoic Era, the coniferous forests shifted southward and reached their present location probably in the end of the Tertiary Period.

SOME VIEWPOINTS ON THE PHYLOGENY OF ABIETACEAE

No one today doubts either the close relationship between the conifers *Abies, Picea, Tsuga, Larix, Cedrus*, and *Pinus* or their origin from common ancestors. Taxonomists are combining all these genera into a single family Abietaceae, recognizing, with this name, the priority of *Abies* in the whole group. Less definite, and more misleading, is the formerly used family name Pinaceae. The genus *Pinus* cannot be considered as a type for the whole group, but is rather a specialized side line in the evolutionary trend of *Proto-Abies*.

The common origin of the Abietaceae from a single ancestral group is further demonstrated in the close relationship of coniferous rusts, which are deeply rooted in the main genera of these conifers (see above).

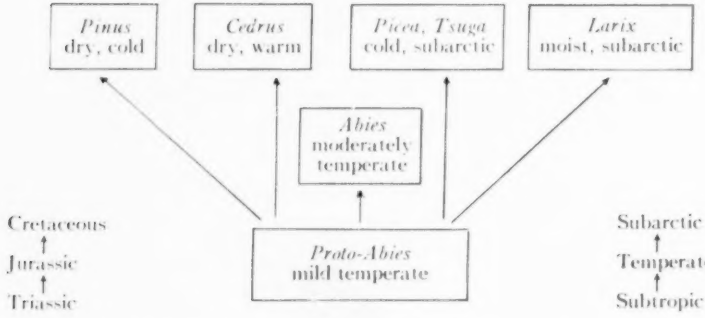
The phylogeny of the conifers has already been considered by many students and described according to the rich flora of their

fossils by E. Irmscher, R. Kräusel, W. Studt, W. Gothan, E. W. Berry, R. Florin and several other paleontologists. The author of this article studied the evolution of conifers in connection with the phylogeny of their rust fungi. Some preliminary reports about this work are published in several former papers (Leppik **8**, **9**, **10**, **11**, **12**, **15**).

If the ancestral group of recent Abietaceae is provided with the provisional name *Proto-Abies*, the following preliminary sketch may be outlined according to the research records mentioned above.

TABLE III

HISTORICAL DEVELOPMENT OF ABIETACEAE



If we restrict the use of the name *Proto-Abies* only to a definite phylogenetical group, which gave rise to the recent family of Abietaceae, several concrete conclusions can be made about this ancestral group according to existing genera and their petrified remnants. Aside from their individual differences, all existing genera of Abietaceae have many common traits that they inherited from their ancestors, i.e. from *Proto-Abies*. All existing genera of Abietaceae inherited also from the *Proto-Abies* their rust fungi, a group of highly specialized parasites, which have accompanied their hosts since their early beginning. The phylogeny and biological specialization of the rust fungi therefore help to trace the historical pathway of conifers, and to establish the relationships between different groups of their host plants. The different soral types of the rust fungi indicate further the special climatic conditions that are responsible for their appearance (see above).

According to these considerations the place of origin of *Proto-Abies* could be located on the northern hemisphere where all recent Abietaceae still live. There is clear evidence that the historical development of Abietaceae was an evolutionary trend among the conifers from a subtropic area toward the boreal one. This trend is also reflected in the development of a protective covering in the sporulating forms of accompanying rust fungi (see above). Another trend was directed from moist soils toward the dry climate.

For *Proto-Abies* can therefore be assumed a mild temperate or subtropic area with well-established climatic seasons. This can be concluded from the well-protected sori and heteroecism of *Sorospora*. A further indication of the climatic seasons may be the annual rings of some Protoabietinae. That the annual rings are caused by growing seasons, interrupted by winter and not by drought, can be concluded from the well-protected winter spores of *Sorospora*.

According to these prerequisites, the equatorial tropic formed a climatic barrier toward the south, which neither *Proto-Abies* nor its phylogenetic successors could ever pass over, except in high mountain areas.

According to geological records the whole territory of recent temperate Asia, Europe, and America has had presumably during the whole Mesozoic Era a tropical climate. The area of ancient *Proto-Abies* can therefore be assumed to be still far north in the present arctics, where its first remnants may lie hidden under the ice and snow. The phylogeny of the rust fungi and of several other groups of plants and animals points in the same direction. Because of the severe weather conditions, hardened plants and trees returned from time to time to more southern provinces, where they may also appear in paleontological records. These fugitives from the arctic can now be traced according to their accompanying rust fungi, whose spore forms indicate their northern origin.

Another assumption about the place of origin of the recent fir family somewhere in the paleotropic mountains of Eurasia is less evident. Neither Abietaceae nor their rusts contain montane types; these are common to the Cupressaceae and *Gymnosporangium* (Leppik **9**, **14**).

To be sure, *Proto-Abies* has, in ancient times, had several related groups with which it has had common, but still earlier ancestors, probably from the group of Prototaxodiaceae. These intermediate forms between Abietaceae, Taxodiaceae, or Araucariaceae, respectively, are arranged by R. Kräusel and W. Eckhold to an indefinite group of Protoabietineae. Of *Proto-Abies* would be a definite phylogenetic group of Protoabietineae, containing the ancestors of recent Abietaceae.

Some of these groups of Protoabietinae could have been infected with *Sorospora*-related rusts. There has been found an aecium fossil on *Glyptostrobus*, which is very similar to our aecial prototype.

Some of the ancient Protoabietineae also reached the southern hemisphere. *Cupressinoxylon Kräuseli* Eckh. from Argentine in Jurassic, *Pityoxylon* Kurtz from Patagonia in Cretaceous, *Planoxylon Hectori* Stopes from New Zealand in Cretaceous, and an uncertain find from Australia by von Ettingshausen, are some examples. But all these ancient conifers lived outside of the *Proto-Abies* area, became extinct without further successors, and cannot therefore have any direct phylogenetical relationship with our recent *Abietaceae*.

The time when the *Proto-Abies* first appeared may be assumed to be in some part of the Triassic Period, from which fossils of Protoabietinae are found in several parts of the northern hemisphere.

There is a remarkable similarity in the phylogenetic development of Abietaceae and their rusts, which fact can be best seen according to the corresponding TABLES I, II, III and FIGS. 1, 2 in the text.

THE HISTORY OF CONIFEROUS RUSTS

The historical pathway of the rust fungi begins with the lowest forms on ferns, continues its course over gymnosperms to the lower angiosperms, and finally reaches its destination on the upper groups of angiosperms (see FIG. 2). On this long road, the conifers play an important role in connecting the lower rusts on ferns, and their highest forms on angiosperms (encircled area on FIG. 2). This main phylogenetic trend of rust fungi is clearly exposed in their biological specialization. It can also be traced back phylogenetically and holo-

genetically. Some of the oldest fern rusts still live on their paleozoic host groups and have also preserved their ancient forms of spores. On the contrary, the medieval rusts prefer to inhabit the gymnosperms; whereas the modern forms of these fungi are exclusively distributed on angiosperms. The relative phylogenetic age of

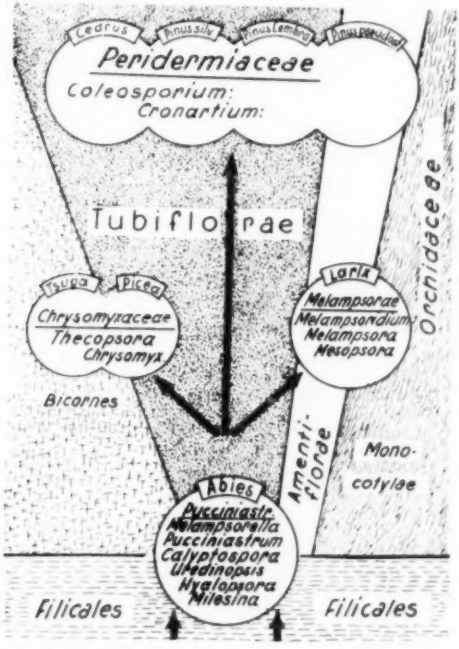


FIG. 5. The advancement of biological specialization of the coniferous rusts and their distribution among the different groups of angiosperms. In shaded sectors are pointed the phylogenetic groups of flowering plants which are inhabited by coniferous rusts.

certain groups of rust fungi can now be determined according to several hologenetic ladders, and some cases of unilateral pleophagia and their irreversible biological specialization (Leppik **8**, **10**, **12**, **15**).

The evolution of coniferous rusts is strictly dependent upon the historical development of their host plants. Every new group among the host plants provides a new opportunity for the rusts, and the evolution of the Abietaceae provides the pathway on which the coniferous rusts have to evolve. The comparative study of

the phylogeny of the conifers therefore helps to solve some of our problems (Leppik **11**).

The rich flora of petrified fossils among the Abietaceae enable one to connect the phylogeny of both conifers and their rusts with the geological time table. Uredineae sorosporae will probably be one of the first groups among the rust fungi whose phylogeny can be established with descriptive methods and whose history can be provided with some chronological dates (see FIG. 3).

On conifers, a number of old rust forms have survived which enable us to reveal the phylogenetic threads between some older groups of these fungi. It is very remarkable that on the fir trees of the genus *Abies* are found the *oldest* heteroecious rust forms, which still preserve their teliospores on ferns as *Milesia, Uredinopsis* and *Hyalopsora* (FIG. 1).

Other coniferous rusts: Chrysomyxaceae on related *Picea* and *Tsuga,* Peridermiaceae on *Pinus* and *Cedrus,* and Melampsoraceae on *Larix* all reveal characteristics of more advanced forms and evolve their teliospores on angiosperms.

This fact indicates that *Abies* should be considered as one of the phylogenetically oldest genera in its family, which viewpoint is, however, not in accordance with paleontological records. The petrified fossils of *Pinus* and *Picea* are known from the Triassic and Jurassic Periods, whereas these of *Abies* only since the Cretaceous. The late appearance of *Abies* in the paleontological records may be explained by the fact that fir cones are extremely fragile and do not produce petrifications. But to identify the fir-tree fossils only by wood remnants is very uncertain. Therefore, other indirect ways must be used to determine the phylogenetic history of the fir tree.

Another indication of the old age of the fir tree may be the unilateral pleophagia (polyphagia) between the rusts on genus *Abies* and numerous fern plants of family *Polypodiaceae.* By the irreversible development of biological specialization of rust fungi (Leppik **14**) it is impossible to imagine that so many rusts from numerous fern genera have joined together to form their aecia on *Abies.* More reliable is the opposite supposition that a group of rust fungi from *Abies* invaded many representatives of *Polypodiaceae,* in

which case the genus *Abies* must be considered phylogenetically older than the Polypodiaceae.

Some more indications for the phylogenetic development of coniferous rusts have been discussed in former articles (Leppik **10, 11, 12**).

An attempt is made in FIG. 3 to sketch the phylogeny of the whole group of Uredineae sorosporae. In the Jurassic Period *Sorospora* was split into several new strains and groups. Some of these groups: Pucciniastreae and Chrysomyxeae preserve their main characteristics and their way of biological specialization. Other groups have a clear inclination towards different ecological conditions on specialized hosts.

Peridermiaceae (Coleosporieae and Cronartieae) on *Pinus* and *Cedrus* are specialized for extreme dry, and Melampsoraceae on *Larix*, for a moist climate. They lost their contact with ferns and have found new hosts for the teliospores, from different groups of angiosperms.

THE BIOLOGICAL SPECIALIZATION OF CONIFEROUS RUSTS

The study of biological specialization of rust fungi among some groups of host plants enables us to determine the period when these hosts first were infected by rusts during their phylogenetic development.

These periods of infection can be further determined by observing when the concerned host groups have split into new forms. If there are enough fossils for study, as in the case of Abietaceae, the periods of infection and the time of splitting into new forms can be connected with the geological time table and the relative age of studied groups can thus be determined.

These comparative studies can disclose most valuable facts about the phylogeny of both rust fungi and their host plants, and can provide some new viewpoints for scientific criticism.

Some points in the phylogenetic development of coniferous rusts which can be connected with a geological timetable (FIG. 3)

1. An ancestral group of blister rusts, which formed its aecia on a group of ancient conifers, can be assumed to have existed in Perm and Triassic about 240,000,000 years ago. Teliospores have presumably been formed on ferns.

2. *Sorospora* lived on ancestors of *Abies* (*Proto-Abies*), and ferns during the Triassic and Jurassic periods in the northern hemisphere (see above).
3. The splitting of *Sorospora* into new forms can be accounted for by the formation of new phylogenetic groups among the *Proto-Abies* in the Jurassic period, some 200,000,000 years ago. This point can be assumed only approximately according to fossils, which show well differentiated groups of Abietaceae since the beginning of the Cretaceous Period.
4. The separation of *Colcosporium* from Cronartiae can be more accurately dated chronologically to the Jurassic period, when *Pinus* was split into the subgenera *Haploxylon* and *Diploxylon*. *Cronartium* is evidently older; its numerous species inhabit both subgenera of *Pinus*. *Colcosporium* is restricted to the subgenus *Diploxylon*, but invades also *Cedrus*.
5. *Melampsora* and *Melampsoridium* have existed as independent groups since the splitting of the Betulaceae into *Betula*, *Populus* and *Salix* in the Cretaceous period, about 150,000,000 years ago.
6. The formation of *Thecopsora* occurred during the separation of *Picea* from *Abies* in Cretaceous.
7. The separation of *Melampsorella* from *Hyalopsora* occurred during the formation of Caryophyllaceae and Boraginaceae in Cretaceous.

THE HOLOGENESIS OF RUST FUNGI

All genetically related groups of organisms, species, genera, families, etc., no matter how heterogeneous they are or where they live, are united in a *phylum*. The genesis and historical development of a phylum is called *phylogeny;* it provides the essential foundation for taxonomy.

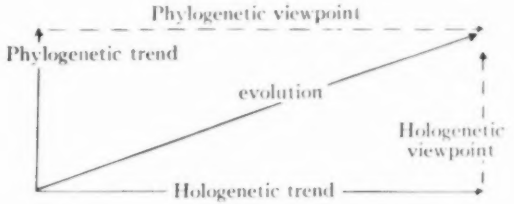
A phylum, however, cannot exist alone, because its life and evolution is dependent upon other groups and upon its environment. In fact, a number of species from different phyla, even from different kingdoms, live contemporaneously in the same environment, forming certain associations, communities, biocoenoses or other combinations. No matter what their phylogenetic origin is, nor where they come from, they have to live together and adjust themselves to each other.

Since the early beginning of the organismic world, animals have been dependent upon plants for their source of food. But plant life is conceivable only by the coexistence of animals, who prepare the food for the plants and serve other essential functions such as pollination, seed distribution, etc. Most phyla of organisms are not only biotically interdependent, but such interdependence is an evolutionary causative agent of prime importance. The inter-

relationships of organisms really force them to live and evolve together as a *biotic whole* or *holon*.

According to a proposition of F. Merkenschlager, the genesis and historical development of the biotic whole may be called *hologeny*. It provides the essential basis for *typology* (Leppik **15**).

Considered in this way, the historical development of every organism is governed by two different groups of factors: *phylogenetic* and *hologenetic*. Both act independently of each other and are attempting to enforce the evolution of the organism into different directions, as follows:



The evolution of the organism really is the resultant of these two components (Leppik **15**).

In our case the hologenetic trend of the rust fungi is dependent upon the evolution of their hosts. Their existence is impossible without host plants and the evolutionary pathway of their hosts is compulsory for the rusts.

Phylogenetically, rust fungi show remarkable stability. Their phylogenetic characteristics, for example basidiospores, are therefore very constant. However, they have to follow their fast-evolving host plants and adjust themselves to different climatic conditions, from the extreme tropic to the extreme arctic. This need for permanent change makes rust fungi hologenetically very plastic. They have to form new types of spores and adjust their sori to different climatic conditions.

Descriptive hologeny is mainly concerned with the study of the principle of interrelationships between main groups of phyla, i.e. the interdependence which binds organisms inseparably to each other. These relationships are: saprophytism and parasitism,

symbiotism and antibiotism, synagonism[1] and antagonism, mutualism and commensalism, etc. In many cases the organisms are dependent upon each other simply as producers and consumers. Analogically with the relation between phylogeny and taxonomy, hologenetic studies provide the foundation for *organismic typology* (**16**).

THE PHYLOGENETIC CHARACTERISTICS OF RUST FUNGI

The most typical phylogenetic characteristics of the whole order of rust fungi are their peculiar way of forming the basidium, bearing single-celled basidiospores, and a constant alternation of generations. These characteristics are constant for the whole order and do not change much during the evolution of these fungi. Their history is long, presumably covering more than 300,000,000 years.

The tender basidia and basidiospores of rust fungi presumably were formed in the tropics during the carboniferous period of the Paleozoic Era. Their formation demands a moist tropical climate with a high temperature. According to Gäumann (**5**, p. 546), the basidia of *Jola javensis*, from the related order of *Auriculariales*, are formed in the damp mountain forests of West Java with an optimal air temperature of 18° to 26° C. and a relative humidity of not less than 90 per cent. These conditions prevail in the rainy season on the moss carpet between the undergrowth, in the late morning to afternoon. After an hour in free air, with a relative humidity of 70 per cent or a temperature below 15° C., the fructification stops.

The rust fungi preserve their tender basidiospores and their tropical way of formation even after the long journey from the tropics to the arctic. The relative moisture and temperature, necessary for the formation of basidiospores in the arctic, differs only by some few degrees from that of the tropics. To survive in the arctic, the rust fungi had to develop several new forms of spores and sori, some with very thick cell-walls. But presumably every arctic rust can survive, where its host turns back to the tropics. These secondary tropic rusts are highly confusing to the taxono-

[1] *Synagonism* (opposite to antagonism) is a special case of mutualism, a symbiotism where the partners are not bound bodily (Leppik **15**, p. 11).

mist, but offer an excellent opportunity to trace the phylogenetic pathway of both the rusts and their host.

THE HOLOGENETIC CHARACTERISTICS OF RUST FUNGI

One of the most plastic spore forms is the teliospore of rust fungi. In a cold climate they have to fill the ecological task of hibernation and have to adjust themselves to extreme conditions. The evolutionary trend of teliospores can therefore not be directed only by inside phylogenetic forces, but is governed by outside hologenetic factors. By their restricted phylogenetic value the teliospores deliver perfect characteristics for tracing the hologenetic pathway of rust fungi. In smaller groups the hologenetic characteristics of teliospores are fixed phylogenetically and can be used for identification of genera.

Aeciospores are characteristic of the heteroecious rust fungi of the colder and drier climate. The single-celled aeciospores are stable, but their sori are adjusted to protect immature spores against drought. They provide very good characteristics for judging climatic conditions and hologenetic tendencies of the rust fungi.

THE IRREVERSIBILITY OF BIOLOGICAL SPECIALIZATION

A new rust form adjusts itself regularly to some new group of host plants, which is phylogenetically younger than the host plants of its origin.

This rule was first observed by W. Tranzschel and confirmed by P. Dietel and Leppik (**14**). Both workers, Tranzschel and Dietel, used this rule to predict the unknown partners for the life cycle of heteroecious rusts. In fact they could establish a number of aecidial hosts and add greatly to our knowledge of the life cycles of several rust fungi.

From Tranzschel's rule can be concluded the irreversibility of the biological specialization of rust fungi (Leppik **14**). When a new fungus form is adjusted to a new group of host plants, its successive generations do not return to their old host plants. This rule is most valuable for the phylogenetic studies of both rust fungi and their hosts. In the heteroecious rust fungi, the irreversibility of specialization leads to the peculiar "hologenetical ladder," as

was first demonstrated by *Melampsora* (Leppik **12**), and then by *Gymnosporangium* (Leppik **9**, **14**) and is also evident in Pucciniaceae (Leppik **8**).

The evolution of the rust fungi is closely connected with an exactly fixed irreversible alternation of generations, as follows:

haplophase → dicariophase → diplophase
P C R

Each of these phases ends with a cytological change of nuclei: plasmogamy (P), cariogamy (C) or division of reduction (R). In *autoecious* rusts, both the haploid (gametophyte) and dicarioid (sporophyte) thalli live on the same host plant; in *heteroecious* forms each generation has a different host (see Gäumann **8**).

TABLE IV

HOLOGENETIC LADDER OF MELAMPSORA (AFTER LEPPIK, 1948)

Hologen. phases of *Melampsora*	Evolution of biological specialization of *Melampsora*	Alternation of generations
Auto-Melampsora	● Teliospores on angiosperms	Sporophyte
Neo-Melampsora	○ Aeciospores on angiosperms	Gametophyte
Salico-Melampsora	● Teliospores on Salicales	Sporophyte
Pino-Melampsora	○ Aeciospores on Pinaceae	Gametophyte
Proto-Melampsora	● Teliospores on ferns	Sporophyte

By regular evolution the heteroecious rust fungus cannot change both its hosts at the same time. One generation must keep its old group of hosts until the other generation is well established to a new host. Then the first generation frees itself from the old hosts and chooses some phylogenetically younger group of plants for its new host.

In this way, the rust fungus with its two generations, sporophyte and gametophyte, climbs the hologenetic ladder of evolution step by step. A sketch of a hologenetic ladder of *Melampsora* is made in TABLE IV and FIG. 4.

It is possible to connect the single steps of a hologenetic ladder with a geological time table and reconstruct a hologenetic time table for certain groups of rusts. Several ladders of different rust

groups may be united to form a permanent hologenetic system (Leppik **12**).

Using hologenetic ladders of different rust groups in connection with the phylogeny of their host plants, it is possible to construct a schedule of the historical development of a whole group.

FIG. 4, small circles (○) refer to aecia, black dots (●) to teliospores. The dots within circles (⊙) point to rusts with both aecia and teliospores on the same host plant.

Some subgeneric groups of *Melampsora* are marked with solid and broken lines and may be characterized as follows:

——— = Urediniospores rounded, teliospores subepidermal.
- - - - - - - = Urediniospores rounded, teliospores subcuticular
▬▬▬ = Urediniospores elongated, teliospores subepidermal.
. . . . . = Urediniospores elongated, teliospores subcuticular.

The studied species of *Melampsora* are marked with numbers as follows: 1. *abieti-caprearum* Tub., 2. *arctica* Rostr., 3. *larici-epitea* Kleb., 4. *larici-caprearum* Kleb., 5. *larici-pentandrae* Kleb., 6. *larici-populina* Kleb., 7. *medusae* Thuem., 8. *larici-tremulae* Kleb., 9. *farlowii* Arth., 10. *albertensis* Arth., 11. *medusae* Thuem., 12. *pinitorqua* Rostr., 13. *ribesii-epitea* Kleb., 14. *ribesii-viminalis* Kleb., 15. *ribesii-purpurea* Kleb., 19. *allii-fragilis* Kleb., 20. *amygdalinae* Kleb., 21. *allii-salicis albae* Kleb., 22. *allii-populina* Kleb., 23. *rostrupi* Wagn., 24. *pulcherrima* (Bubak) Maire, 25. *magnusiana* Wagn., 26. *orchidi-repentis* (Plowr.) Kleb., 27. *evonymi-caprearum* Kleb., 28. *lapponum* Lindf. 29. *hirculi* Lindr., 30. *vernalis* Niessl, 31. *alpina* Juel., 32. *cathartici* Buchheim, 33. *lini* (Ehrh.) Lév., 34. *liniperda* Palm, 35. *euphorbiae-dulcis* Otth., 36. *euphorbiae* (Schum.) Cast., 37. *euphorbiae-gerardiana* W. Mueller, 38. *euphorbiae-silvaticae* (DC.) 39. *helioscopiae* (Pers.). This list is incomplete.

FIG. 4 shows the further advancement of biological specialization of the coniferous rusts and their distribution among the different groups of angiosperms.

SOME CONCLUSIONS

The idea of studying the phylogeny of the rust fungi in connection with their host plants is by no means a new one. W. Tranzschel (**1918**), A. Mordwilko (**1922**) and P. Dietel (**1936**) took some of the first steps in this direction and a number of other workers in biology have pointed to the great value and necessity of such studies. The main obstacle that hindered this work was the absence of a definite phylogenetic basis and the lack of an applicable method for these studies. The problem that arose was how to combine and coordinate the phylogeny of two such genetically dis-

tant groups as the rust fungi and the higher plants. The absence of fossils makes the restoration of the past history of the rust fungi extremely difficult.

A new way has been found to study the phylogeny according to *"living fossils"* (Gäumann), which are abundant among the rust fungi. The rusts are, in fact, a very special group among plants, and have preserved their ancient traits beside those newly acquired characteristics of modern types.

The *hologenetic studies* help to establish the relative phylogenetic age of different interrelated groups of organisms, even in cases of distant phylogenetic relationships. The *irreversibility of biological specialization* of rust fungi (Tranzschel, Leppik) enables us to establish the *hologenetic ladders* (Leppik), which reflect the real evolutionary trends and progress of the groups they represent. The ladders of different groups may be united to form a permanent hologenetic system which can be connected with a geological time table. In several cases of *unilateral pleophagia* (Leppik **11**) it is also possible to determine the relative phylogenetic age of different groups of host plants which the rusts inhabit. In this way it is possible to trace the very obscure history of the rusts and to reconstruct main outlines to the phylogeny of one of the most difficult and diversified groups of fungi. The method is simple and easily applicable, whereas the results can be controlled and improved at any time according to living specimens.

The hologenetic method of consideration is not based upon presumptions or hypothesis, but is a very reliable way of describing objects and facts exposed in nature.

Both phylogeny and hologeny have the same aim: to picture the historical development of organisms according to present forms, or using for this purpose petrified remnants. But both these trends have different viewpoints.

Phylogeny studies the historical development of a certain genetically related *taxonomic group*, from the phyletic standpoint of view. *Hologeny* traces the development of interrelations of genetically not related *typological units*, from the holetic viewpoint of the biotic whole or holon.

The perception of these two disciplines offers two different methods in the contemplation of the historical development of

organisms that lived in the past and cannot be seen or described directly.

The accuracy and completeness of our picture of the actual course of evolution is enhanced when we view it hologenetically as well as phylogenetically.

To be sure, only some few groups of rust fungi have been studied up to this time from the hologenetic viewpoint. The whole picture is still incomplete and the evolutionary schemes as they are presented in this article need corrections as soon as the other rust groups are studied.

The purpose of this article is to present some results for further criticism and to win more workers to this new field.

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SHORT-SEASON ADAPTATIONS IN THE RUST FUNGI[1]

D. B. O. Savile[2]

(WITH 3 FIGURES)

Intensive botanical work in northern Canada in recent years, particularly by members of this Division, has led to an increase in our knowledge of the parasitic fungi in the arctic and subarctic regions. Each year since 1948, specimens of rusts have been obtained both through intentional collecting and through the scrutiny of all northern phanerogamic specimens acquired by the Division. Although such specimens are of great value in adding to our knowledge of distribution and host range, they inevitably lack much of the data that would enable a mycologist to understand the biology of the species concerned. This fact was forcibly impressed upon me upon examining the rusts secured from four northern areas in 1948. I was, therefore, glad of the privilege to serve during the three succeeding summers as botanist with Northern Insect Survey parties at Great Whale River, Que., on the southeast coast of Hudson Bay, at Chesterfield Inlet, Keew., on the northwest shores of Hudson Bay, and at St. Anthony near the northern tip of Newfoundland.

Great Whale River is a tree-line station with typical arctic tundra along the coast but a rapid transition into spruce forest in valleys a few miles inland.

Chesterfield Inlet is a truly arctic station, although about 220 miles outside the arctic circle. It is about 300 miles beyond the accepted tree line, and probably nearly 200 miles from the last outlying clumps of spruce. The area consists of severely glaciated granite gneiss with a maximum altitude of less than 100 feet for several miles inland, in a bleak, wind-swept region with a very

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severe climate. Southern elements in both flora and fauna are fully as scarce as at some stations much further north; several ferns are absent that occur abundantly in more rugged terrain some 300 miles northeast in the Melville Peninsula; and many plants are extremely depauperate.

St. Anthony lies in the part of the northern peninsula of Newfoundland that is north of Hare Bay. All this area is within seven miles of the sea. It is in consequence wind-swept and the trees do not attain great size; but it enjoys a much longer frost-free season than Great Whale River. Perhaps because of these circumstances the flora shows a curious mixing of southern and high arctic elements. *Abies balsamea*, which is absent from Great Whale River, is abundant, as are several weeds that have been introduced at Great Whale River but have failed to become established.

These three stations may fairly be taken to represent tree line, the arctic and the northern transitional forest respectively. The latter is in some respects a mosaic of tundra and boreal forest, rather than a true vegetation zone; but the transition is in many places of great depth and the forest usually poorly developed and not wholly typical of the boreal zone. Its recognition accordingly seems desirable. From field observations at these stations it has been possible to determine the approximate northern limits for rusts of different types and to study some of the adaptations that permit various species to persist despite a short season. Rusts with such adaptations are by no means confined to the north, but their predominance there is overwhelming. The adaptations are sufficiently varied and striking to merit consideration in some detail.

The distribution data of some species used as examples are naturally considerably at variance with those given in existing floras. Details of distribution will be given in later taxonomic papers.

PUCCINIACEAE

In temperate regions a substantial proportion of the rust fungi have a life cycle nearly or exactly like that of a heteroecious, long-cycled *Puccinia*, shown diagrammatically in FIG. 1. In the spring

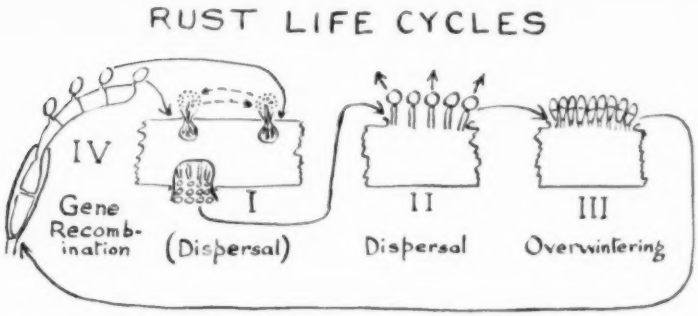

Fig 1.— Heteroecious, self-sterile, long-cycled Puccinia

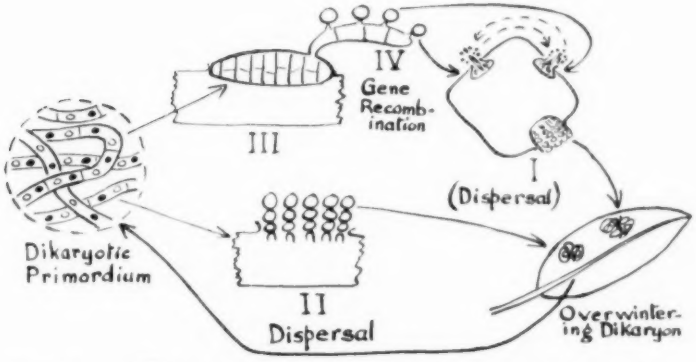

Fig. 2.— Typical Chrysomyxa with telescoped life cycle

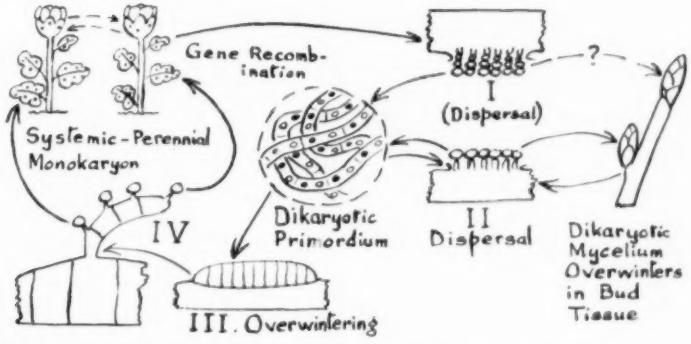

Fig. 3.— Variable life cycle in Melampsora epitea

the overwintered telia germinate, the basidiospores produce pycnia on the alternate host, the dikaryon is re-established and the aecia are formed. These stages function mainly to effect gene recombination, but the aecia have a secondary function in aiding dispersal. The urediniospores are conidia, serving to disperse and propagate the rust.

In southern Canada and the northern United States there is time for several uredinial generations; consequently the aecial and telial hosts may be separated by a considerable distance, and the association between them is not always obvious. As we go farther north the time available for uredinial generations is progressively decreased, and lower prevailing temperatures greatly slow down the rate of development. The decreased opportunity for build-up of the population makes survival impossible unless the alternate hosts are very close together. A number of grass and sedge rusts occurred at St. Anthony, but their spread was usually limited to two to six feet from the aecia and there appeared to have been an average of only about one full generation of uredinia before the formation of telia. At Great Whale River several *Carex* rusts occurred, but generally there was only a partial uredinial generation overlapping the formation of telia, and infection was usually limited to within a foot or so of the aecia. The only obligately heteroecious grass rust at Great Whale River was *Puccinia praegracilis* var. *connersii*. As has already been indicated (**5**, **6**) the few uredinia and telia formed were almost contiguous with the aecia. It thus appears that tree line is about the limit for heteroecious, long-cycled species. It is probable that in a succession of favorable years such a rust may invade the barrens for a hundred miles or more, only to be forced back by the first poor season.

An example of an unsuccessful invasion of the barrens was noted at Chesterfield Inlet in 1950. A scattering of aecia was found on the leaves of *Ranunculus pallasii* in a shallow pond, but only on plants contiguous with the aquatic grass *Colpodium fulvum*. The rust is suspected to be a strain of *Puccinia rubigo-vera* specialized upon these two plants of similar habitat, and its normal range will probably prove eventually to be in the northern transitional zone, much of which is still botanically *terra incognita*. In

1949, a favorable season in much of the arctic and subarctic, uredinia were probably formed in quantity and enough reached Chesterfield Inlet to give a trace infection of telia. In 1950 the spring was late and on 9 August the aecia were just starting to open; most were open by 19 August, but by then the growing season was coming to a close. Growth of all plants seemed to have stopped by the end of the month and the *Colpodium* plants were ripening without any sign of infection. No further observations could be made, but it seems inconceivable that any telia could have matured under the cool conditions with frequent frosts that prevail in September.

The virtual suppression of uredinia as tree line is reached is not an adaptation, of course, but an inevitable and serious consequence of the environment. However, one characteristic is shared by *Puccinia praegracilis* and the unnamed *Aecidium* that may be interpreted as a short-season adaptation. In both these rusts there is a complete lack of pycnia. Presumably both species are self-fertile. The loss of pycnia actually represents more than the attainment of self-fertility; it is tantamount to the loss of all chance of cross-fertilization. This serious sacrifice could probably be borne only in well-adapted, successful species. It is likely to occur most frequently where it has some compensating value. In these northern species it has a clear advantage in reducing the time needed for completion of the life cycle. *Puccinia sorghi*, for example, needs about five days to mature the first aeciospores after mixing of the pycnial nectar, in the laboratory at 71° F. In the field, at about 55° F, it would probably require about ten days; and at tree line, with mean temperatures of about 45–50° F, the requirement might be about 15 days. Fifteen days saved out of a growing season of about three months may allow some spread by urediniospores when it would otherwise be impossible.

There are various other modifications of the heteroecious Puccinia type of life cycle, which, although also found in temperate zones, have evident advantages in the north. Several heteroecious rusts live over in the uredinial state on plants whose leaves remain green throughout the winter, obviating the need for proximity of the aecial host. *Puccinia poae-sudeticae* is sufficiently abundant on *Poa* spp. and *Trisetum spicatum* at various locations near tree

line to justify the belief that it may spread some distance into the barrens. *P. bistortae* overwintered at St. Anthony mainly as telia, producing aecia abundantly on *Conioselinum chinense* and sparingly on *Ligusticum scothicum;* whereas Great Whale River it overwintered abundantly as uredinia, and no aecia were seen on the plentiful *L. scothicum.* This rust is common on *Polygonum viviparum* far into the arctic in areas where no aecial hosts occur. Possibly the abrupt ending of the growing season in the north has a physiological effect on the leaves that is conducive to continued production of uredinia, whereas in the south a gradual senescence promotes the change to telia. Other species in this category are *P. caricis,* which persists on certain *Carex* spp. near tree line, and *P. obscura,* which was recently found on overwintered *Luzula* leaves from Nome, Alaska. This process is not short-cycling as the term is properly understood, but is a sort of optional short-circuiting of the cycle. It is interesting to note that this is essentially the same device as that which permits cereal rusts to persist in warm climates where teliospores cannot germinate.

The autoecious full-cycled rust presumably has a slight advantage over its heteroecious counterpart, since a higher proportion of its basidiospores and aeciospores may be expected to fall on the appropriate plant. One example is *Phragmidium arcticum,* which was abundant enough on *Rubus acaulis* at Great Whale River to suggest that it might survive considerably farther north; a second is *Uromyces armeriae,* which was heavy in two colonies at the same station. Closely related to the last type are the autoecious brachycyclic species, best represented by *Puccinia hieracii,* which was plentiful at Chesterfield Inlet on *Taraxacum pseudonorvegicum.*

A further advantage may be confered by the systemic habit, as is shown by the occurrence of *Uromyces lapponicus,* with systemic-perennial aecia but localized telia, on *Oxytropis* spp. near or beyond tree line at various stations. The initiation of systemic infection appears always to require quite exacting conditions, but in this species the disadvantage seems to be offset by the prompt production of masses of aeciospores and a guaranteed survival even in years of very little plant growth. In point of fact telia of *U. lapponicus* were surprisingly scarce at St. Anthony and none could be found at

Chesterfield Inlet; and it is suspected that beyond tree line they often fail to develop.

Finally, the arctic and subarctic yield a number of short-cycled species of *Puccinia* and *Uromyces*, many of them widespread and abundant. These may be of the firm-pedicelled Eupuccinia type, in which the teliospores must germinate *in situ*, or of the Bullaria type with fragile pedicels, in which the spores may germinate when detached. The latter type enjoys some advantage over the former, because the teliospores may assist in dispersion. In each of these types the mycelium may be localized or systemic. The firm-pedicelled, systemic species are represented by *Puccinia holboellii*, which is fairly abundant on *Arabis* and *Draba* north at least to tree line. The firm-pedicelled, localized species are represented by *P. pulsatillae*, which occurs appreciably beyond tree line, *P. arenariae*, which occurs frequently far into the arctic, and *P. grindeliae* and *P. millefolii*, which extend at least to tree line. *P. drabae*, which is relatively common at Chesterfield Inlet and has been received from other arctic stations, and *Uromyces phacae-frigidae*, which is known from Alaska and Yukon, are examples of the systemic Bullaria forms. The best represented microcyclic forms are the localized Bullarias, several of which occur far beyond tree line. Examples are: *Puccinia blyttiana* (*P. ranunculi*) on *Ranunculus pedatifidus*; *P. heucherae*, *P. pazschkei* and *P. saxifragae-tricuspidatae* on *Saxifraga* spp.; *P. eutremae* and *P. oudemansii* on Cruciferae; and *P. mertensiae* on *Mertensia maritima*. All the short-cycled species listed lack pycnia and are presumably self-fertile. The majority attack rosette-forming or prostrate perennials (annuals are almost absent from the arctic), in which the leaves largely remain attached until the following season. This provision guarantees that the new season's leaves and flower stalks develop in close proximity with the sori of the previous year. In the arctic none of these species seems to produce more than one generation of spores in the year, although some, such as *P. arenariae* and *P. heucherae*, are freely leptosporic farther south. Here we have the ultimate simplification of the life cycle, with persistence assured by the close proximity of the new host tissues to the source of inoculum, but little opportunity for extensive spread. It is easy to understand, therefore, why in-

fection is often very heavy in a single clump of a host plant but absent from clumps a few feet away.

The preponderance of short-cycled rusts in mountain areas has been discussed by Arthur *et al.* (**2**), but the attempt by the same authors to show north to south distribution of micro- and macrocyclic species in North America was less successful, partly because the zones were too broadly delimited and partly because arctic and subarctic data were largely lacking. When data for the arctic are essentialy complete the microcyclic species will be found to outnumber the macrocyclic substantially; and such macrocyclic species as do occur will almost all prove to have some auxilliary means of overwintering.

CHRYSOMYXA

The genus *Chrysomyxa* provides interesting examples of short-season adaptations, as has already been indicated (**4**). FIG. 2 shows the life history of a typical, long-cycled species such as *C. ledicola*. All the stages found in a long-cycled *Puccinia* are adhered to, but the times of year for their formation differ considerably. These differences are possible because the telial host is always a plant with evergreen foliage. The rust overwinters on this host as a dikaryotic mycelium rather than as a telium. In the spring renewed growth gives rise to telia and uredinia nearly simultaneously. Usually the telia slightly precede the uredinia, but a single sorus sometimes produces both spore forms simultaneously. By the time that the sori are mature the new leaves of both *Picea* and the telial host are emerging from the winter buds; and the former become infected by the basidiospores, the latter by the urediniospores. Thus the processes of gene recombination and of dispersal and multiplication proceed simultaneously, telescoping the life cycle. In the south there may be additional spread by secondary uredinia, but in the north no uredinia are seen on the new leaves. The infecting aeciospores and urediniospores simply produce overwintering mycelia. Two representatives of this group, *C. empetri* and *C. ledicola*, persist in the dikaryotic state several hundred miles beyond tree line.

There are several modifications of the typical *Chrysomyxa* life cycle. *C. pyrolae* is systemic and full-cycled. The aecial stage is

systemic, but not perennial, in cones of *Picea*, and the dikaryotic mycelium is systemic and perennial in *Pyrola* and *Moneses*. The perennial mycelium assures the persistence of the rust, but the critical conditions for infection limit its spread. Aecia are seldom abundant and the species is scarcer than several others in most of its range. It is boreal to subarctic in distribution. *C. woronini* has a similar life history except that it has dropped the uredinial stage. The aecial stage is systemic in the young shoots of *Picea* and the dikaryotic mycelium forms perennial witches' brooms on *Ledum groenlandicum* and *L. palustre*. The only North American records are from near Dawson, Yukon, Great Whale River, Que., and St. Anthony, Nfld. It was relatively common at St. Anthony, but scarce at Great Whale River. *C. woronini* seems to be primarily a species of the northern transitional forest, but it is unlikely to prove common anywhere but in humid areas. Finally we have the microcyclic species, represented in North America by *C. weirii* on *Picea* and *C. arctostaphyli* on *Arctostaphylos uva-ursi*. The latter is limited to the boreal zone by the distribution of its host. *C. weirii* may eventually be recorded near tree line, but all records to date have been relatively southern. We must conclude that in *Chrysomyxa* the typical, full-cycled species can cope as well with a short season as any modified type that has yet arisen.

HYALOPSORA ASPIDIOTUS

Another mechanism that achieves the simultaneous, or at least overlapping, production of the different stages is seen in *Hyalopsora aspidiotus*. This rust produces amphispores, which germinate in spring, giving rise to uredinia and telia more or less simultaneously on the fronds of *Dryopteris disjuncta* in early or midsummer. Although the teliospores germinate immediately, the aecia do not develop on *Abies* until the next season or next but one; but they finally open in midsummer. Thus the cycle is so telescoped that all spore stages occur more or less simultaneously, yet without sacrificing genetic plasticity. Although *D. disjuncta* is not a dominant fern at St. Anthony, several colonies of *H. aspidiotus* were found. Aecia and uredinia sporulated freely in late July. I feel

confident that this species will eventually be found north to the limit of *Abies balsamea* (about Hamilton Inlet, Labr.), and it may well occur still further north in the uredinial state.

MELAMPSORA EPITEA

Our last and, perhaps, most remarkable examples of short-season adaptations are to be found in the willow rusts of the genus *Melampsora*. This group is a great complex of intergrading forms, a detailed taxonomic treatment of which must be postponed for some time. As far as the study has progressed it appears impossible to follow Arthur's (1) segregation of the complex into four species. The disposition even of many southern collections is difficult; but the numerous northern forms completely obliterate the presumed distinctions, and the situation is complicated by the fact that in the north aecia are rarely produced. At present it seems advisable to follow Jørstad (3) and call the whole complex *Melampsora epitea* (Kunze & Schmidt) Thuem., regardless of aecial host. As our knowledge increases it will probably be possible to set up several varieties on the basis of host relationship and small morphological distinctions. The only alternative seems to be to make species of the forms on almost every willow.

FIG. 3 and the following discussion apply particularly to the rust on *Salix arctica* and *Saxifraga rivularis*, in which all the illustrated complexities of the life cycle were demonstrated at Chesterfield Inlet. It is, however, fully applicable to any other arctic forms that possess an aecial stage, since it is fairly certain that *Saxifraga* spp. are the only aecial hosts beyond tree line; but for many forms no aecial connection has been demonstrated, and in some the ability to produce one may have been lost.

The telia form in late summer and germinate in the spring. The basidiospores infect the *Saxifraga* plants, probably in the apical meristem just as growth starts, and produce systemic-perennial mycelia. Because pycnia are produced in abundance before the aecia appear it seems reasonable to assume that the mycelium is monocaryotic at least in the aerial parts, but no cytological study has been made. The aeciospores infect *Salix* leaves, producing a dikaryotic mycelium from which may develop uredinia or telia. The

uredinia produced from aecia probably do not often sporulate early enough to produce many secondary uredinia but some of the spores sift or grow under the scales of the newly formed winter buds, forming an overwintering mycelium in the embryonic leaves or catkins. The mycelium grows with the bud tissues in the spring and produces large or multiple uredinia early enough in the season to permit the production of secondary uredinia and of telia in some quantity. This device is very similar to that by which the dikaryon over-winters in *Chrysomyxa*, but only uredinia are produced in the spring. Some aeciospores may also possibly give rise to overwintering mycelia, but probably the majority are shed before the willow terminal buds are formed. Here we have three methods of overwintering, and three interlocking cycles, in a single rust. First, the monokaryon can persist year after year in the absence of *Salix*, although with no spread. Second, the dikaryon can persist in the winter buds, producing uredinia and permitting spread but no genetic change. Finally, the full cycle, with gene recombination and spread, is possible when the two hosts are contiguous.

The ability of *M. epitea* to overwinter in the willow buds was mentioned by Jørstad and was obvious in some of the specimens received in 1948. The fact that the mycelium is systemic-perennial in *Saxifraga* is also of prime importance, but it seems to have received no recognition in the literature. Observations at Great Whale River convinced me that tree line was about the limit for the conventional long-cycled rust. It was disconcerting to receive, soon afterward, heavily rusted *Saxifraga oppositifolia* from Cornwallis I., roughly a thousand miles beyond tree line. The habit of *S. oppositifolia* is such that the systemic condition was not obvious in these specimens, although it is clear to me now. The puzzle was not resolved until infected plants were seen in the field at Chesterfield Inlet.

Although *M. epitea* was moderately common on *Saxifraga rivularis* and occurred sparingly on *S. cernua* and *S. oppositifolia*, at least 90 per cent of the rust in *Salix* colonies at Chesterfield Inlet originated from the winter buds. At Great Whale River and St. Anthony all rust appeared to result from bud infection. The available specimens suggest that the aecial stage of the forms on arctic

willows occurs most frequently north of tree line, probably because that is where the susceptible saxifrages are most abundant. Specimens of *M. epitea* in its various forms have come in abundance from almost all northern stations from which phanerogamic collections have been received. It has been seen on *Saxifraga* from 75° N., and on *Salix* from about 82° 30′ N. in northern Ellesmere I., almost the northernmost land in the world. In the vicinity of Chesterfield Inlet it occurred on seven out of thirteen *Salix* spp. and was abundant on several. Clearly it is a most successful species, and the modifications in its life cycle have played no small part in its success.

ACKNOWLEDGMENTS

The personal observations drawn upon for this paper were made during field work with the Northern Insect Survey sponsored by the Defense Research Board of Canada. The majority of the pertinent collections made by others were also taken by members of Northern Insect Survey parties. Without this sponsorship any real understanding of the rust flora of the Canadian arctic would still be impossible.

SUMMARY

The northern limit for heteroecious, self-sterile, long-cycled species of *Puccinia* and *Uromyces* is approximately tree line. Self-fertility and short-cycling enable many species to persist in the arctic on hosts of suitable habit. In *Chrysomyxa,* overwintering of dikaryotic mycelium in evergreen leaves permits simultaneous development of uredinia and telia, and also makes the rust independent of the aecial host. *Hyalopsora aspidiotus* overwinters both by amphispores and by mycelia in the aecial host, thereby producing aecia, uredinia and telia nearly simultaneously. *Melampsora epitea* overwinters as a systemic-perennial monokaryon in *Saxifraga* and as a dikaryon in *Salix* buds, as well as by telia, thus being able to survive even when the sexual part of the life cycle cannot be completed.

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LARGE-BROWN-SPORED HOUSE-ROT FUNGI IN THE UNITED STATES

ROSS W. DAVIDSON AND FRANCES F. LOMBARD[1, 2]

(WITH 4 FIGURES)

Merulius lacrymans Jacq. ex Fr. has never been considered a common or extremely destructive fungus in North America. It is recorded by Burt (**4**) as occurring here and has been encountered occasionally in houses. *Poria incrassata* (Berk. & Curt.) Burt, a closely related fungus and originally placed in the genus *Merulius*, is more common as a cause of house decay (**1, 9, 10, 13**). In 1946 Buckland (**3**) listed and illustrated cultures of *Merulius* sp. isolated from brown rot in Western red cedar trees, and Bier *et al.* (**2**) reported *Merulius* sp. from similar rot in fir in 1948 in western Canada. *Merulius* sp., similar to the one described by Buckland, has been obtained frequently from spruce and balsam fir trees in eastern Canada and Maine. These findings have led to a more intensive study of cultures of the large-brown-spored species of *Merulius*.

DIFFICULTY OF IDENTIFICATION

Sporophores of the fungi considered here are seldom present in association with decay in trees and are almost never included with wood specimens sent in from decay in buildings. The first fresh sporophore of *Poria incrassata* encountered by the senior writer was on the under side of a large Douglas-fir log in the woods. It was not recognized as this species because in the fresh condition it was not brown or dark in color. It was a well-developed, light gray, poroid layer and the spores were only slightly colored when

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[2] The authors are indebted to Dr. J. D. Diller and others for decay specimens and to other laboratories for some of the cultures used.

mounted in KOH solution. Usually when fruiting is found in buildings it is old and at least partially dried up and very dark.

In contrast, *Merulius lacrymans*, when obtained in good fruiting condition, is bright red or rust brown from the copious spore deposit. Under the microscope, spores are uniformly golden brown. However, other thinner species of the large-brown-spored *Merulius* group are difficult to separate from *M. lacrymans* unless fruiting bodies are in good condition. Spores of several species are similar in size and color. Sporophores of *Coniophora puteana* (Schum. ex Fr.) Karst. (*Coniophora cerebella* Pers.) might also be confused with *Merulius* species if not obtained in excellent condition.

After studying cultures of many wood-rotting fungi, the writers have been able to readily distinguish cultures of *P. incrassata* and *C. puteana* from *Merulius* spp. Evidence is presented to show that cultures of *M. lacrymans* may also be distinguished from cultures of a common thin species, *M. americanus* Burt. It is believed that the species of *Merulius* obtained from eastern Canada and Maine may be *M. americanus*.

CULTURAL CHARACTERISTICS [3]

Key to Cultures

1. Growth fast [4] (more than 90 mm. diameter in two weeks), mycelium buff to brown ..... *Coniophora puteana*
1. Growth medium (60 to 90 mm. diameter)
   2. Mat irregularly silky or with radiating silky segments, mycelium white ..... *Poria incrassata*
   2. Mat uniformly fluffy, somewhat lobed, with or without zonations, mycelium white or white with some scattered yellow color ..... *Merulius americanus*
1. Growth slow (30 to 50 mm. diameter), mat raised fluffy, mycelium white or with scattered yellow color *Merulius lacrymans*

[3] Explanation of methods used in studying wood-rot fungi in culture has been published (7).

[4] Growth is at a room temperature of about 25° C on two per cent Fleischmann's diamalt agar.

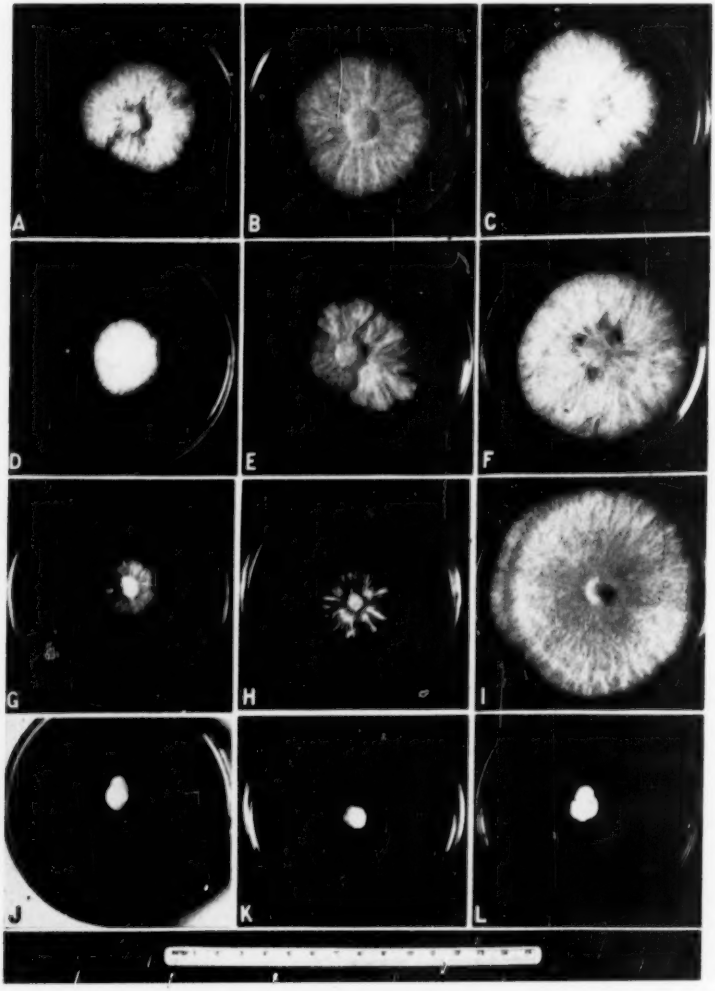

FIG. 1. One-week-old cultures grown on 2 per cent malt agar at room temperature. *A* to *C*, *Merulius americanus*, from Maryland and Georgia; *D* to *F*, *M. americanus*, from Maine and eastern Canada; *G* and *H*, *Poria incrassata*; *I*, *Coniophora puteana*; *J*, *Merulius lacrymans*, from Europe; *K* and *L*, *M. lacrymans*, from the United States.

CONIOPHORA PUTEANA (Schum. ex Fr.) Karst.

(FIG. 1, I; FIG. 2, G and H; FIG. 3, A)

Key pattern.[5] B-O-F-1-11-16.

(See published description (**5**).) Twenty-eight isolates.

MERULIUS AMERICANUS Burt

(FIG. 1, A–F; FIG. 2, A and B; FIG. 3, B; FIG. 4, A)

Key pattern. B-O-M-1-10, A-O-M-1-10, and B-O-M-1-10-16.

Growth characteristics. Growth medium, forming a mat 32–58 mm. in diameter in 7 days, 60–90 mm. in 14 days; white, occasionally with faint "citron yellow"[6] color on the inoculum or scattered around it at 7 days, usually with pale "citron yellow" or "primrose yellow" (rarely "buff-yellow" to "orange cinnamon") on or near the inoculum or localized on zonation lines in the central zone by 14 days; generally with thin fluffy growth mounded over the inoculum, mat proper intermediate to raised, uniform to margin, of radiating silky-downy hyphae at first, later fluffy-downy mycelium almost obscures the skeinlike silky-downy hyphae underneath except near the inoculum, with or without zonations, occasionally with conspicuous colorless guttulation drops on or near the inoculum, margin distinct, even or lobed, finely fimbriate; some isolates staining the agar a yellow color, which shows through the mycelium somewhat; odor strong, penetrating, disagreeable; negative oxidase reaction,[7] growth at 7 days on gallic acid medium 14–22 mm. in diameter, no growth to trace on tannic acid medium.

Hyphal characteristics. Hyphae straining, thin-walled, 2–7 (– 10)μ in diameter, with abundant clamps on large and small

[5] The key pattern has been explained previously (**7**, pp. 9–13). The first letter represents color of mat (A, white; B, white, then yellow or brown; C, yellow, etc.). The second letter of the key pattern represents positive (P) or negative (O) oxidase reaction. The third letter indicates growth rate. The numbers indicate presence of certain microscopic features such as clamps, chlamydospores, conidia, etc. The key pattern is useful in arranging the fungi, based on their characteristics in culture.

[6] Colors in quotation marks according to Ridgway (**14**).

[7] Fungi causing brown carbonizing rot in wood usually give no reaction when grown on agar containing gallic or tannic acid whereas white rot fungi cause a dark color reaction when grown on such media (**6**).

hyphae, sometimes irregularly thickened septations and walls in some empty hyphae, and occasionally more or less globular staining swellings or masses seen on some submerged hyphae; crystals octohedrals and many small angled thin plates or lumps.

Test-tube cultures. Mycelium fluffy-downy on agar slant, white, silky-downy against test tube below slant with a few to numerous small more dense strands of hyphae; yellow to "orange cinnamon" above; some cultures having numerous guttulation drops.

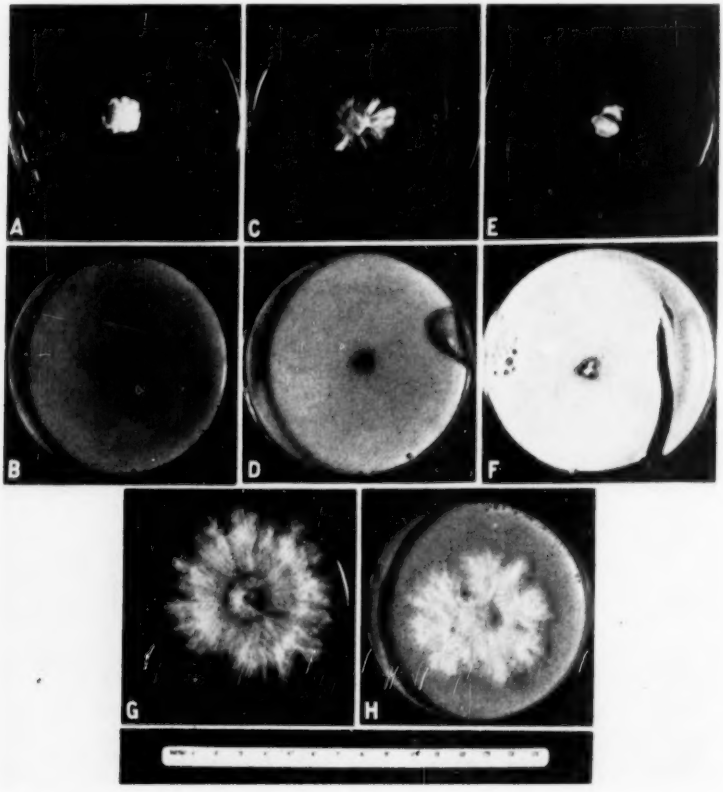

FIG. 2. One-week-old cultures on gallic and tannic acid agars grown at room temperature. *A*, *C*, *E*, and *G*, gallic acid; *B*, *D*, *F*, and *H*, tannic acid. *A* and *B*, *Merulius americanus*; *C* and *D*, *Poria incrassata*; *E* and *F*, *Merulius lacrymans*; *G* and *H*, *Coniophora puteana*.

FIG. 3. Twenty-eight-day-old test-tube cultures on malt agar. *A, Coniophora puteana; B, Merulius americanus; C, Poria incrassata; D, Merulius lacrymans.*

Type of decay. Brown rot of coniferous wood. Twenty-five isolates.

Remarks. The above description specificially applies to cultures obtained from a species that fruits commonly on pine logs in the woods in the vicinity of Beltsville, Md. These sporophores seem to fit Burt's description of *Merulius americanus,* but it is believed that *M. himantioides* Fries and *M. brassicaefolius* Schw. may not be specifically distinct. Also it appears that *M. silvester* as described by Falck is the same as *M. americanus.* For the time being these will have to be regarded as synonyms of one species or as a species complex that will require much more detailed study before they can be distinguished culturally. At present it is not clear which name should be used if they are to be regarded as a single species.

The various isolates from spruce and balsam fir in eastern Canada and Maine show considerable variation in growth rate but appear to be applicable to the same species as the one collected on pine logs at Beltsville. The sporophores developed on pine logs in late fall (October and November). If the eastern Canada fungus is the same, sporophores should also be common there on old infected logs in late fall. Sporophores have been collected several times under houses during the winter season.

PORIA INCRASSATA (Berk. & Curt.) Burt

(FIG. 1, G and H; FIG. 2, C and D; FIG. 3, C; FIG. 4, B)

Key pattern. A-O-M-1-10, A-O-M-1-4-10, B-O-M-1-10, and A-O-M-1-10-16.

Growth characteristics. Growth moderately rapid, forming a mat 23–38 mm. in diameter in 7 days, 62–71 mm. in 14 days; white, occasionally with small patches of "naples yellow" to "straw yellow" on the inoculum or sparingly around it at 14 days; appressed to intermediate, uniformly fine thin short downy to the margin of growth or, more generally, with small skeins arising near the inoculum or in the central zone or at the margin of growth and extending as radiating, fast-growing, silky mycelium, in some isolates ending abruptly in short raised isolated tufts and in other isolates spreading in silky fanlike sectors within mats of otherwise uni-

formly fine downy growth at 7 days, becoming more or less appressed in the central zone and raised at the margins, with the skeins and fanlike sectors (where present) still distinct at 14 days, zonate or azonate, fragile, adherent, sparse cottony aerial mycelium over the inoculum, margin either thin, even, and almost indistinct or fimbriate and distinct where silky mycelium present; odor medium to strong pungent; negative oxidase reaction, growth at 7 days on gallic acid medium 17–28 mm. in diameter, no growth to trace on tannic acid medium.

Hyphal characteristics. Hyphae staining, thin-walled, 1.7–4.7 (−8)μ in diameter, averaging about 2.5–3.5μ, with abundant simple clamps on both large and small hyphae, rarely double clamps, occasionally in some isolates submerged hyphae have elongated thin-walled swellings that stain readily; oidia, few to many in the areas of slow-growing downy mycelium, of varying lengths; crystals large and medium octohedrals and thin plates.

Test-tube cultures. In 4 weeks mycelium yellow to buff or light brown and appressed on agar slant around inoculum (with agar showing through), white dense silky strands or dense mat developing lower down between glass wall of tube and agar; sometimes small dense powdery cushions of mycelium developing around the inoculum or at upper end of agar slant.

Type of decay. Causes brown rot of both hardwood and coniferous wood in houses and other wooden structures, and very occasionally infects logs in the woods. Fifteen isolates.

Remarks. It is present occasionally although not abundant in houses but is very destructive when present under favorable moisture conditions. This fungus is probably the only one of the group included in this paper that has not been reported in Europe. It is more prevalent in the eastern United States but occurs in the West also.

MERULIUS LACRYMANS Jacq. ex Fries

(FIG. 1, J–L; FIG. 2, E and F; FIG. 3, D; FIG. 4, C)

Key pattern. B-O-S-1-10 and B-O-S-1-10-16.

Growth characteristics. Growth slow, forming a mat 11–19 mm. in diameter in 7 days, 27–40 mm. in 14 days; white, usually with

patches of "citron yellow" color over or around the inoculum block; growth on inoculum raised, of densely mounded fine fragile downy mycelium extending upward toward the lid of the dish and laterally to almost hide the surface mat at first, merging gradually into a

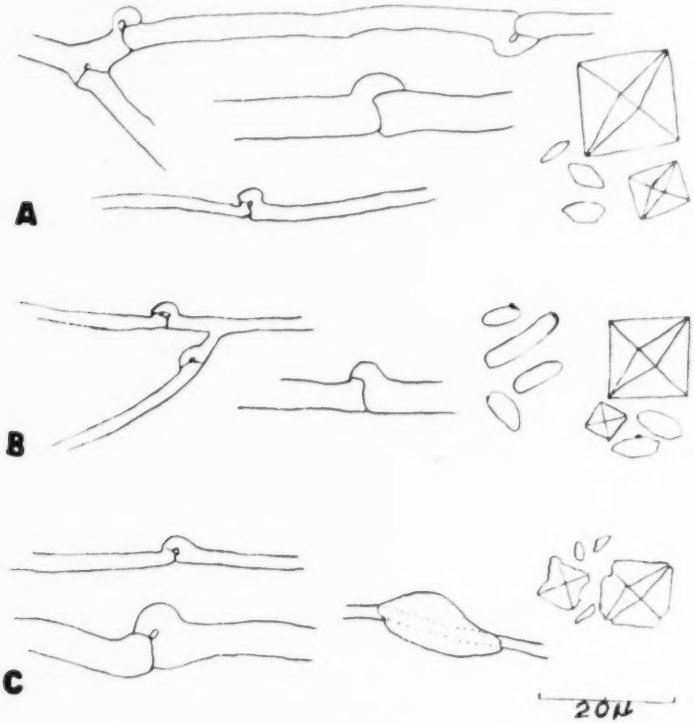

FIG. 4. *A, Merulius americanus:* hyphae and crystals. *B, Poria incrassata:* hyphae, oidia, and crystals. *C, Merulius lacrymans:* hyphae, staining mass, and crystals.

fluffy more downy-cottony less raised growth toward the margin, fragile but slightly firm underneath, azonate, margin distinct, fimbriate, even or lobed; odor faint or none; negative oxidase reaction, growth at 7 days on gallic acid medium trace to 13 mm. in diameter, 0 to 13 mm. on tannic acid medium.

Hyphal characteristics. Submerged and superficial hyphae staining, thin-walled, 2–7(−9.5)μ in diameter, with numerous

clamps on large and small hyphae; occasionally submerged hyphae with slight swellings or masses that stain; crystals numerous, octohedrals and small angled plates. According to Nobles (**12**), haploid cultures have no clamp connections and numerous oidia.

Test-tube cultures. In four weeks growth at ordinary room temperature is rather limited, covering agar slant and growing only slightly onto upper part of agar cylinder, uniform fine fluffy-downy mat, white or with some "citron yellow" color.

Type of decay. Brown rot of coniferous wood. Three isolates.

Remarks. This fungus is not as common as *Poria incrassata* in this country but does occur occasionally in buildings in which moisture conditions are favorable and the temperature relatively low.

TEMPERATURE RELATIONS

As shown in TABLE I, three of the four fungi studied have intermediate and the other one (*Merulius lacrymans*) low temperature requirements for optimum growth. The species have not shown

TABLE I

FUNGUS GROWTH ON MALT AGAR, MEASURED AT THE END OF ONE WEEK AT CONSTANT TEMPERATURES

Name of fungus	Average diameters of growth at constant temperatures									
	18° C	20° C	22° C	24° C	26° C	28° C	30° C	32° C	34° C	36° C
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
Coniophora puteana	76*	82	87	90+	90+	90+	66	14	trace	0
Merulius americanus	34	30.6	41	45.6	47.5	47.5	33.5	23	trace	0
Poria incrassata	20	24	26	43	42	40	35	20	trace–0	0
Merulius lacrymans	27	28.5	21	23	16	trace	0	0	0	0

* Mat diameters are averages of several growth tests.

a very wide range of temperature requirements among the different isolates. This was the case with *M. lacrymans*, although only one European and two American isolates were available for study. All of the cultures show a rather wide optimal temperature range.

The principal point of difference between these results and those of Humphrey and Siggers (**11**) is that their one European isolate of *M. lacrymans* had an intermediate temperature requirement.

It is possible that such variation could result from differences between strains of a species. If further careful studies show such wide variation to be common, separation by temperature requirements alone would be questionable. So far in our experience this has not been shown to be true and any proof of wide variation within a species must be verified by accurately identified sporophore cultures.

DISCUSSION

The four species can be differentiated readily at four days of age on malt dishes. *Coniophora puteana* makes the fastest growth and the mat is cream-colored, intermediate to raised, and mainly downy to downy-cottony composed of fine or coarse strands of hyphae radiating from the inoculum block. *Merulius americanus* is next in growth rate and the mat is white, with or without a tinge of lemon yellow near the inoculum, and is mostly appressed, composed of bunches of silky-downy radiating and ascending hyphae with thin but highly raised growth over the inoculum. *Poria incrassata* is slightly slower growing than the above species, making scant growth over the inoculum block; the mat is appressed and composed of very short, slightly ascending, downy mycelium with the characteristic dense radiating silky growth just beginning at the margin of the mat. *M. lacrymans* is the slowest in growth rate and the mat is white, consisting mainly of raised fluffy dense growth over the inoculum, with scant growth on the surface of the agar.

Two of the four fungi described in this paper, *P. incrassata* and *C. puteana,* are quite destructive in houses and other wooden structures in the United States. The other two, *M. lacrymans* and *M. americanus,* are present only occasionally in buildings, and the latter is not known to cause extensive destruction. *M. americanus* and *C. puteana* are common on trees or logs in forested areas, whereas the other two are not known to be common in the forests.

All but *P. incrassata* may be common in Europe. An examination of Falck's 1912 paper (**8**) suggests that his three large-brown-spored species may be the same as the three studied here. Our *M. lacrymans* should be his *M. domesticus* and *M. americanus* may be the same as *M. silvester.* Most certainly the *C. puteana* in

houses in this country is the same as the fungus Falck described under the name *C. cerebella*.

It is probable that there are additional large-brown-spored *Merulius* species in this country besides the two mentioned in this paper but up to the present time the writers have not encountered any that is clearly culturally distinct.

SUMMARY

Cultures of *Merulius lacrymans* have been distinguished from cultures of other wood-rotting fungi and from cultures of the common species *Merulius americanus*. The two species of *Merulius* may be separated by differences in growth rate and mycelial mat characteristics when grown on 2 per cent malt agar in 90 mm. Petri dishes. *M. lacrymans* grows best at a temperature of about 18° C and does not grow at 28° C. *M. americanus* grows best at 26° C, grows well at a temperature of 30° C, and will even make a slight growth at 34° C. These *Merulius* species have been encountered a few times in houses.

Coniophora puteana and *Poria incrassata* are two species causing destructive decay in houses in this country; they also have large brown spores. However, *C. puteana* is a much faster growing fungus than the *Merulius* species and *P. incrassata* may be differentiated from the other species by its mat and type of growth. Temperature requirements for *C. puteana*, *P. incrassata*, and *M. americanus* are quite similar.

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SPECIES OF SYNCHYTRIUM IN LOUISIANA. VIII. NEW SPECIES

MELVILLE T. COOK

(WITH 51 FIGURES)

The species of the genus *Synchytrium* cause the formation of galls or abnormal growths on the leaves, petioles, stems and sometimes on flowers of the host plants. Most species of *Synchytrium* attack but one species of host plant. The external characteristics of some of them and the fact that they are specific enable the student to identify many species with some degree of certainty and without difficulty. The descriptions of 43 of 95 species reported from the northern hemisphere are incomplete but they very definitely belong to this genus. Some of the characters used in the descriptions, such as size and color of fungus, are of very little value; the size is variable even in the mature stages and the colors are variable with age and are practically the same in all species. The zoospore is hyaline; the young fungus becomes light yellow soon after infection and then gradually orange, red and sometimes black with age.

In some species the sori can be seen as yellow or orange spots without difficulty, while in some others they can be seen only by the aid of a hand lens.

The fungus in some species stains so deeply that the structure cannot be seen without destaining. In these cases the stain usually disappears from the host tissues far in advance of the stain in the fungus. In some species the fungus does not overstain readily. The shape and structure of the galls is more definite and valuable for descriptions and identification than any characters of the fungus. In this paper, more attention is given to the galls than to the fungi. The descriptions of the 43 species which are incomplete do not contain any information concerning the sporangia. These data are very difficult to obtain in some cases, because the fungus becomes separated from the host tissues and falls out of

the cavity before the formation of the sporangia. The galls are very conspicuous in some species while in others they are very inconspicuous, causing, in fact, scarcely any deformity of the host tissues.

Although the galls are very definite in shape, they may show wide variations. A gall in contact with a vein may be quite different from one that does not have such a contact and the writer has usually classed them as abnormal or deformed galls.

Infection of the trichomes is very rare. The zoospore has been observed to enter the stomata in a few cases but without the formation of a gall. The period of infection is previous to the formation of the palisade. There are a few records in the literature of sub-epidermal infections but the author is inclined to doubt these studies. The infections in all species studied by the writer are in the epidermal cells, usually in the lower epidermis. The writer has never observed infections in sub-epidermal cells. In one species (*S. oxalidis* Cook) overgrowths gave the appearance of sub-epidermal infections, but this was due to overgrowths. The infected host cell reacts very quickly after the penetration by the zoospore. In a few cases the fungus increases in size for a time and then become dormant in the tissues of the host plant (FIGS. 17, 39). This is probably due to a late infection of the host cell which does not react. The cells of the galls are frequently infected when very young; the result is the formation of compound galls.

Infections occur during periods of wet weather, which may be very short. In Louisiana the infections occur in the late winter or spring following rains which may flood areas for a very few hours. The infections occur on very low herbaceous or very young and very small woody plants.

The descriptions of species previously reported by the author have contained descriptions of the sporangia. The sporangia have not been studied in the species reported in this paper because it is necessary for the writer to discontinue this work. However, the data are sufficient for the identification of the species.

The author has described several species of *Synchytrium* collected in Louisiana. In many cases distinct species will be found on different host plants but growing very close together. This

has raised the question as to the possibility of some of these species attacking several different species of host plants. If that is true, some of my species should be reduced to synonyms. Of course, this question cannot be answered definitely without making cross-inoculation experiments, which the writer is not in a position to do at this time. However, we have certain facts to support the writer's contention that most or possibly all of them are distinct species. The literature indicates that of 95 species previously reported, 40 species occur on one species of host plants only, 6 species occur on two species of host plants, 4 species on three species of host plants, 2 species on four species of host plants and one species on six species of host plants. This indicates that most species of *Synchytrium* are specific in their attacks on host plants. The one exception to this conclusion is *S. aureum* which has been reported on 197 species of host plants, but these records are questionable. *S. aureum* is most likely a composite of many species. Furthermore, the writer has shown that in some cases two species of host plant grow in contact and only one species is attacked by the fungus. An example of this is *S. stellaria* which attacks *Stellaria media*, and *S. cerastii* which attacks *Cerastium viscosum*. These two species of host plants are frequently found growing in contact and only one infected. The same has been noted on other species but the observations have not been so numerous. In some species the shape and structure of the galls are so definite as to indicate the species. This is in harmony with the writer's studies on galls caused by insects, many years ago, in which it was found that the structure of the galls depended on the species of insect and not on the host plant.

Synchytrium Parthenocissi sp. nov.

Gallis post contagionem celerrime ortis; in utraque superficie foliorum sitis; per spatium circiter aequale ultra alteram superficiem se extendentibus; colore subviridi ad colorem purpureum; saepimentis leviter demutatis aut absentibus; cavis subsphaericis; foramine claro; 304–760 μ. Fungus cellulam primo sed haud serius implet; 15–248 μ diametro. Hab.: *Parthenocissum quinquefolia* (L.) Planch.

Galls on both surfaces of leaves, nearly spherical; may extend about equal distance on either surface but usually most prominent on lower surface; the palisade is slightly modified; when the pene-

tration is on the upper surface, the palisade is broken, shortened or absent, structure rather dense; cavity nearly spherical; cells around cavity are modified very quickly; greenish to purple in color; sometimes concave above with papillae in center; opening distinct; the gall grows very rapidly and in advance of the formation of the fungus but the leaf begins to thicken very soon after infection. The fungus fills the cell completely at first (FIG. 1) but after a time the cell cavity grows more rapidly than the fungus (FIG. 2). Wall around fungus is very thin. The cell material surrounding the fungus is thick and stains very dark (FIGS. 1, 2).

Similar infections which may be caused by the same species are found on *Ampelopsis arborea* (L.) Desmoulins and *Vitis* sp.[1]

Synchytrium Duchesneae sp. nov.

Gallis in utraque superficie foliorum; insitis aut in superficie foliorum manentibus; 450 × 600 vel 600 × 750 μ. Fungus 95 × 300 μ. Hab.: *Duchesnea indica* (Andr.) Focke. Not abundant. Baton Rouge, Louisiana, U.S.A.

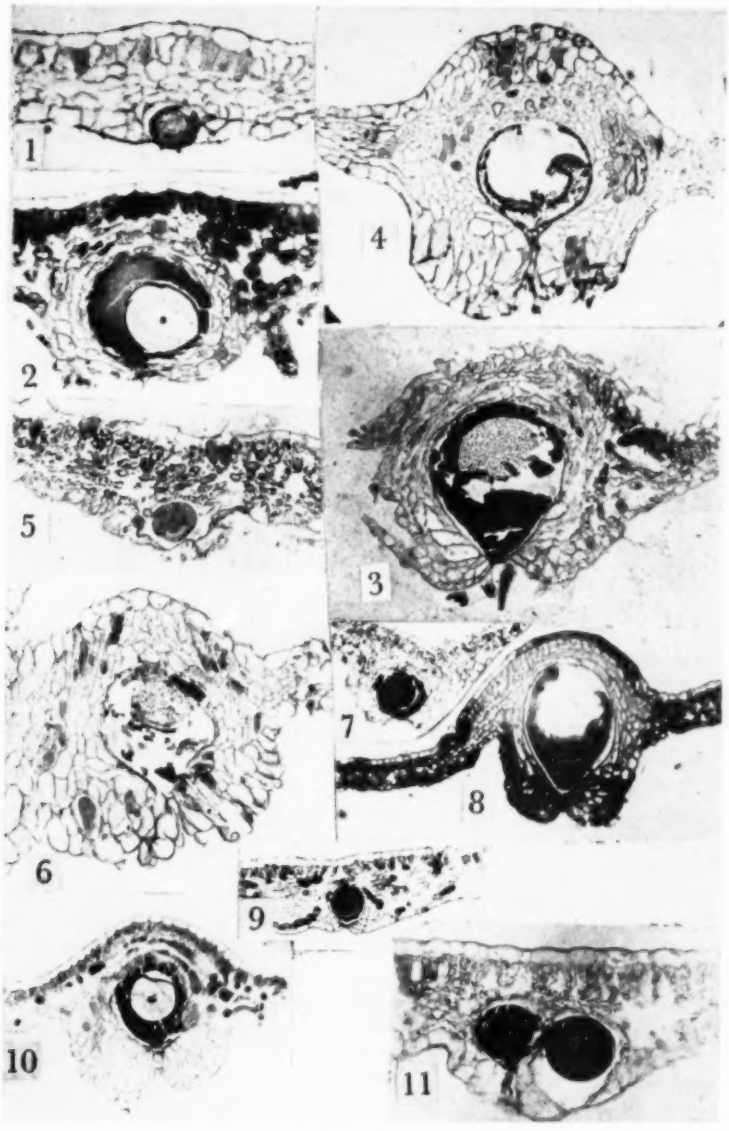
Leaf structure very loose. Galls on leaves and petioles; large, projecting on both surfaces of leaves with opening mostly on under surfaces; may rest on surface of leaf or be submerged in tissues of the host; green; galls grow more rapidly than the fungus; cavity filled by fungus at first but not later; very little modification of cell structure of leaf but similar to *S. Parthenocissi;* surface of young cavity usually spherical; opening very distinct.. Wall around fungus thin. Palisade usually absent in mature gall. FIGS 5, 6.

Synchytrium Corni sp. nov.

Gallis in utraque superficie foliorum sitis; in parte inferiore maxime exstantibus; sparsis; hemisphaericis; viridibus; 180 × 375 vel 525 × 525 μ.

[1] Galls caused by *Phylloxera vastatrix* are common on *Vitis* spp. but are easily recognized.

FIGS. 1–4. *Synchytrium Parthenocissi.* 1. Early infection on lower surface of leaf. Early stage of gall formation. 2. The sorus ready for division; gall of small cells; content of infected cell prominent. 3. Gall composed of small cells. 4. Mature gall; opening distinct. FIGS. 5–6. *Synchytrium Duchesneae.* 5. Early infection. 6. Mature gall. FIGS 7–8. *Synchytrium Corni.* 7. Early infection. 8. Mature gall. FIGS. 9–10. *Synchytrium Asterum.* 9. Early infection. 10. Advanced stage; sorus ready to divide; content of infected cell prominent; gall composed of small cells; palisade intact. FIG. 11. *Synchytrium Epihaleri.* Two infected cells; one on left with well pronounced tube; palisade not injured.



FIGS. 1–11.

Fungus clarissimus, 150 × 250 μ diametro. Hab.: *Cornus Drummondi* Meyer, Baton Rouge, Louisiana, U.S.A.

Leaf structure dense; galls on both surfaces of leaves; sometimes abundant but usually scattered; greenish; hemispherical and most prominent below; concave above with elevation in pit; opening distinct. Palisade undeveloped. Fungus usually showing through on under side. Wall around fungus very thin. FIGS. 7, 8.

Synchytrium Asterum sp. nov.

Gallis parvis; in utraque superficie foliorum; plerumque sphaericis; colore viridi usque ad colorem purpureum; 225 × 330 μ vel 300 × 300 μ. Fungus 105 μ diametro. Hab.: *Aster* sp., Baton Rouge, Louisiana, U.S.A.

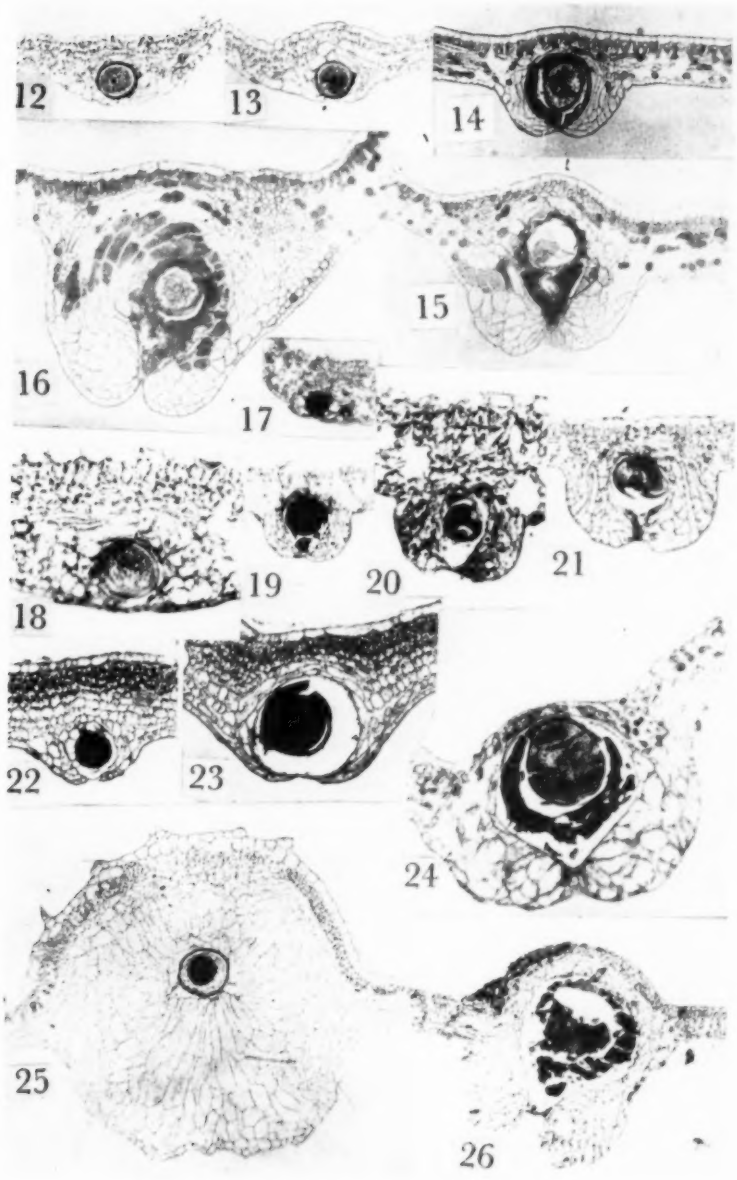
Leaf structure very loose; galls small; on both surfaces of leaves; usually hemispherical; green to purple; opening usually on lower surface; content of host cell very dense and deep staining; wall around fungus thick; plants too young to determine species. FIGS. 9, 10.

Synchytrium Cocculi sp. nov.

Gallis plerumque in una superficie foliorum sitis; basi in textibus hospitis insita; viridibus; 150 × 525 μ. Fungus in folio partim insitus; flavus aut ruber; 60 × 150 μ. Hab.: *Cocculus carolinus* (L.) DC., Baton Rouge, Louisiana.

Leaf structure very loose; galls usually small and on one surface of the leaf or the basal part embedded in leaf; convex on both sides but most prominent on lower surface; spherical on stems; may rest on surface or be partially embedded in tissue; green; cavity connected with the outside by long or short tube. Fungus yellowish to brownish red; wall around sorus very thick. FIG. 11.

FIGS. 12–17. *Synchytrium Cardiospermi*. 12. Very early infection. 13. Slightly advanced. 14. Further advanced, showing substance in infected cell. 15. Well advanced; infected cell cavity pear shaped; content of infected cell prominent. 16. Further advanced. 17. Late infection in which growth of sorus has stopped and gall not formed. FIGS. 18–21. *Synchytrium Ilicicola*. 18. Early infection showing thickening of leaf and formation of wall around sorus. 19. Early stage in gall formation. 20, 21. Advanced stages in gall formation. FIGS 22–24. *Synchytrium Liquidambaris*. Stages in formation of gall. FIGS. 25–26. *Synchytrium Gonolobi*. 25. Advanced stage showing that the gall is very large and the sorus very small. 26. Mature gall.



FIGS. 12-26.

Synchytrium Cardiospermi sp. nov.

Gallis in utraque superficie foliorum sitis, numerosissimis autem in inferiore; 391 × 75 vel 375 × 525 μ. Fungus 27–120 μ diametro. Hab.: *Cardiospermum Halicacabum* L., Baton Rouge, Louisiana.

Leaf structure very loose; galls on either surface of leaf but most abundant on lower; gall starts very soon after infection; cavity pear-shaped in mature galls; opening distinct; fills cavity at first but not later in its development; content of cavity thick; palisade may persist or be only partially developed; green. Wall around fungus very thick (fungus may be quite large and gall quite small). Fungus may become dormant without forming gall (FIG. 17). FIGS. 12–17.

Synchytrium Ilicicola sp. nov.

Gallis numerosis; plerumque in superficiebus inferioribus sitis; in textibus hospitis partim aut omnino insitis; duris, praesertim in stirpe; 150 × 375 μ. Fungus 75–105 μ diametro. Hab.: *Ilex vomitoria* Ait. and *I. decidua*.

Leaf structure loose; galls on leaves mostly on lower surface; hemispherical below and slightly convex above; numerous; sometimes partially or completely embedded in host tissue but usually resting on surface of leaf; green when young; green to dark purple with age; becoming hard with age, especially on stems; surrounding cells may become modified quickly. Wall around fungus thick. Leaf structure slightly distorted. Fungus grows rapidly and fills cavity but grows slower than the cavity after the formation of the gall. Opening to cavity distinct. Fungus may be embedded in leaf without forming gall. FIGS. 18–21.

Synchytrium Liquidambaris sp. nov.

Gallis in utraque sed plerumque inferiore superficie sitis; sphaericis; viridibus; 150 × 150 vel 450 × 450 μ. Fungus 75–300 μ diametro. Hab.: *Liquidambar styraciflua*.

Leaf structure very dense; galls on both surfaces, mostly on lower; usually spherical below and concave above; green; opening very distinct; gall usually larger on the lower surface than on the upper surface; palisade may be destroyed; fungus fills cavity at first but not later; wall around fungus very thick; cell content prominent; fungus may lie in tissue without forming gall. FIGS. 22–24.

Synchytrium Gonolobi sp. nov.

Gallis in utraque superficie sitis; numerosissimis autem in inferiore; hemisphaericis; 600 × 600 μ. Fungus 75 μ diametro. Hab.: *Gonolobus* sp.

Leaf structure very loose; galls on either surface but most abundant on lower; hemispherical; starts immediately after infection and before the palisade is formed; opening indistinct; palisade slightly modified; $600 \times 600\,\mu$; fungus fills cavity for a time but cavity enlarges more rapidly than fungus. Content of infected cell prominent with age. Fungus small as compared with gall; wall around fungus thick. FIG. 25.

Synchytrium Carpini sp. nov.

Gallis raris; in utraque superficie foliorum, numerosissimis autem in inferiore sitis; sphaericis; $210\,\mu$ diametro. Fungus $135\,\mu$ diametro. Hab.: *Carpinus* sp.

Leaf structure loose; galls rare; more or less spherical and prominent on lower surface; opening distinct. FIG. 26.

Synchytrium Clematidis sp. nov.

Gallis in utraque superficie foliorum sitis; sphaericis; 300×400 vel $600 \times 750\,\mu$ Fungus $90 \times 150\,\mu$ diametro. Hab.: *Clematis* sp., Baton Rouge, Louisiana, U.S.A.

Leaf structure very loose; galls usually projecting on one surface or concave above and sometimes below; spherical; opening distinct; sometimes new galls are formed on epidermal cells of older galls. Both fungus and gall grow rapidly. Wall around fungus thick. Content of infected wall very thin. FIG. 27.

Synchytrium Bignoniae sp. nov.

Gallis numerosis in utraque superficie foliorum sitis; sphaericis in petiolis stirpibusque; utraque superficie aequaliter prominentibus; duris, rubris, purpureis, nigris; 300×300 vel $750 \times 1500\,\mu$. Fungus 75–$182\,\mu$ diametro. Hab.: *Bignonia capreolata* L., Baton Rouge, Louisiana, U.S.A.

Structure of leaf very dense; galls abundant on both surfaces of leaves and on petioles and stems; mostly on lower surface of leaves; projecting about equally on both surfaces or larger on side of infection; usually spherical; large and hard; greenish, yellowish, reddish, purple, becoming black with age; cell structure compact in mature galls; cavity almost spherical; tube leading to cavity very small but distinct. Fungus fills cavity at first but not later. Wall around fungus thin. Very abundant. FIGS. 28–30.

Synchytrium Erechtitis sp. nov.

Gallis parvis; numerosis; in utraque superficie foliorum; infra hemisphaericis; in petiolis stirpibusque; 105×150 vel $600 \times 600\,\mu$. Fungus 90–

150 μ diametro. Hab.: *Erectites hieracifolia* (L.) Raf., Baton Rouge, Louisiana, U.S.A.

Leaf structure very loose when young; galls small; abundant on both surfaces; hemispherical below; on petioles and stems; may be embedded in leaf tissue; opening very large. Fungus fills cavity at first, but cavity grows more rapidly than fungus; opening distinct; cell structure compact and with very little modification; 90–150 μ; very pale, becoming yellowish. Wall around fungus very thick. Palisade usually absent. FIGS. 31, 32.

Synchytrium Polygoni sp. nov.

Gallis in utraque superficie foliorum; plerumque numerosis et in racemis; parvis; viridibus; 300 × 300 vel 525 × 600 μ. Fungus 90–195 μ diametro. Hab.: *Polygonum* sp., Baton Rouge, Louisiana, U.S.A.

Leaf structure very dense; galls on both surfaces of leaves; usually abundant and in clusters; small and green; palisade not present in mature galls; host cell material distinct; cavity pear-shaped; opening distinct. Fungus yellow, becoming orange. Wall around fungus thin. FIG. 33.

Synchytrium Callicarpae sp. nov.

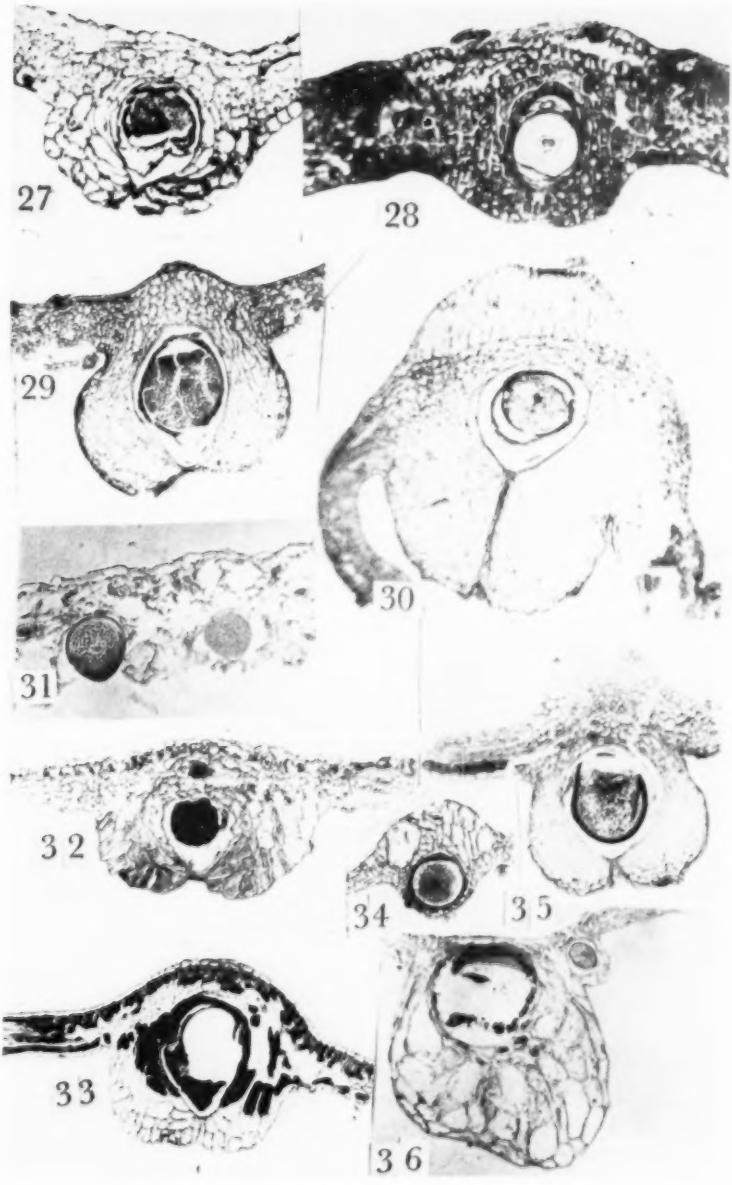
Gallis numerosis; parvis; in utraque superficie foliorum sitis; sphaericis aut elongatis; viridibus; 450 × 450 vel 525 × 525 μ. Fungus 45–180 μ diametro. Hab.: *Callicarpa americana* L., Baton Rouge, Louisiana, U.S.A.

Leaf structure loose; galls numerous, small; projecting on both surfaces of leaves; may be spherical or elongated; usually raised but frequently depressed above; infections start on lower surface but gall usually projects above; green, almost same color as leaves; leaves frequently deformed; in some cases canal to infected cell is distinct; wall around fungus very thick. FIGS. 34–36.

Synchytrium Tecomae sp. nov.

Gallis in utraque superficie sed plerumque in inferiore se extendentibus; viridibus; 300 × 450 vel 450 × 600 μ. Fungus 150 × 450 μ diametro. Hab.: *Tecoma radicans* (L.) Juss., Baton Rouge, Louisiana, U.S.A.

FIG. 27. *Synchytrium Clematidis*. Mature gall. FIGS. 28–30. *Synchytrium Bignoniae*. Stages in the formation of the gall. The sorus is much smaller than the mature gall; canal small and well defined. FIGS 31–32. *Synchytrium Erechtitis*. 31. Early stage showing the formation of the wall around the sorus. 32. Mature gall showing large opening and uninjured palisade. FIG. 33. Synchytrium Polygoni showing mature gall. The palisade is slightly injured. FIGS 34–36. *Synchytrium Callicarpae*. 34. Early stage in formation of gall. 35, 36. Later stages.



FIGS. 27–36.

Leaf structure very loose; galls on upper and lower surfaces; frequently causing a pit in the leaf; most common on lower surfaces; projecting on both surfaces; green; cavity spherical; opening distinct; palisade modified. FIGS. 37, 38.

Synchytrium Mitchellae sp. nov.

Gallis in utraque superficie foliorum sitis; maxima parte in superficiebus inferioribus sparsis; hemisphaericis; viridibus; 150 × 150 μ. Fungus circiter 75 μ diametro. Hab.: *Mitchella repens* L., Baton Rouge, Louisiana, U.S.A.

Leaf structure very loose; galls scattered on both surfaces of leaves, but mostly on lower surface, convex above and concave below; hemispherical; forms very quickly; green; may remain in tissues without causing gall (FIG. 39); fills cavity but cavity enlarges more rapidly than fungus; cell content takes stain very readily. FIGS. 39–42.

Synchytrium Trachelospermi sp. nov.

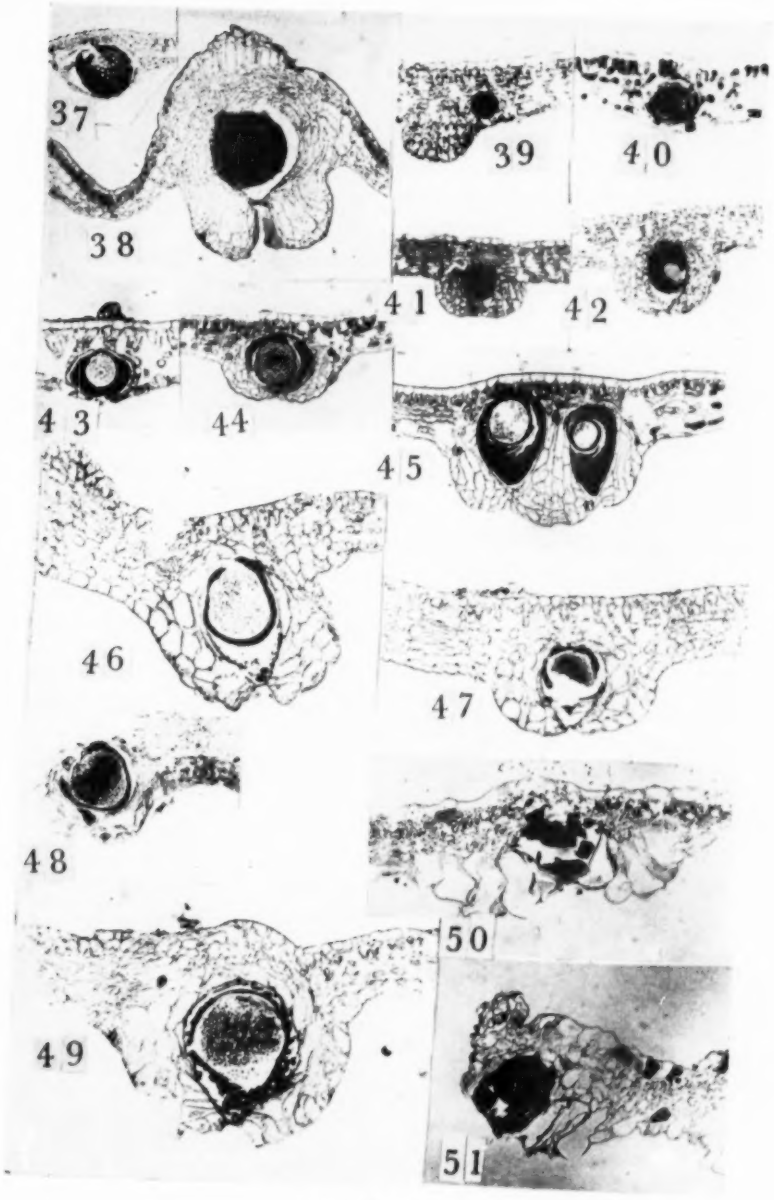
Gallis hemisphaericis; basi in textu insita; viridibus; 150 × 175 vel 600 × 650 μ. Fungus 105–180 μ diametro. Hab.: *Trachelospermum deforme* (Walt.) Gray, Baton Rouge, Louisiana, U.S.A.

Leaf structure loose; galls hemispherical below; convex above; base embedded in the tissues; green; cavity spherical becoming pear-shaped; both cavity and fungus enlarge rapidly; content of host cell dense and deep staining; wall around fungus thick. Very little change in structure of leaf. Host cell content prominent. FIGS. 43–45.

Synchytrium Fraxini sp. nov.

Gallis minimis; maxima parte in superficie inferiore foliorum sitis; colore viridi usque ad colorem nigrum; 300 × 350 vel 600 × 600 μ. Fungus 95–300 μ diametro. Hab.: *Fraxinus* sp.

FIGS. 37–38. *Synchytrium Tecomae*. 37. Early stage in formation of gall. 38. Mature gall. FIGS. 39–42. *Synchytrium Michellae*. 39. An infection without formation of gall. 40. Early stage is in formation of gall. 41, 42. Advanced stage in formation of gall. FIGS. 43–45. *Synchytrium Trachelospermi*. 43. Early stage showing that the sorus has developed very rapidly; the leaf is slightly swollen. 44, 45. Advanced stages in formation of gall. FIGS 46–47. Synchytrium Fraxini. Stages in the formation of the gall. FIGS. 48, 49. *Synchytrium Ulmi*. Early and late stages in the formation of the gall. FIGS. 50, 51. *Synchytrium Violae*. Showing gall composed of large, elongated cells.



FIGS. 37–51.

Leaf structure very loose; galls very small; mostly on lower surface; opening distinct; green to black; wall around fungus very thick. FIGS. 46, 47.

Synchytrium Ulmi sp. nov.

Gallis raris; in utraque superficie foliorum, numerosissimis autem in inferiore inventis; $225 \times 255\ \mu$. Fungus $120\ \mu$ diametro. Hab.: *Ulmus* sp., Baton Rouge, Louisiana, U.S.A.

Leaf structure loose; galls rare; projecting on both surfaces but most common on lower surface; opening prominent; wall around fungus very thick. FIGS. 48, 49.

Synchytrium Violae nov. sp.

Gallis raris; in superficie inferiore foliorum sitis; magnitudine 80×300 vel $80 \times 375\ \mu$ variantibus; compage cellularum laxissima, basi in textu hospitis insita. Fungus circiter $105\ \mu$ diametro. Hab.: *Viola* sp., Baton Rouge, Louisiana, U.S.A.

Leaf structure rather loose; galls usually on lower surface of the leaf and variable in size, ranging from 80×300 to $80 \times 375\ \mu$; the basal part embedded in the host tissue; composed of very long cells; opening very distinct. Substance around fungus very distinct. Fungus about $105\ \mu$ in diameter. FIGS. 50, 51.

Synchytrium globosum Schroeter has been reported on six species of *Viola* in Europe. *S. aureum* Schroeter has been reported on three species of *Viola* in Europe and on one species in Japan. Neither of these species corresponds in measurements or structure with the species found in Louisiana.

The writer wishes to express his thanks to Dr. S. J. P. Chilton for encouragement in these studies, to Dr. C. W. Edgerton and Dr. E. C. Tims for making the photographs and to Dr. P. J. Moorehead for preparing the latin diagnoses.

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SOUTH AMERICAN SPECIES OF PUCCINIA ON SALVIA [1]

JOHN W. BAXTER [2]

(WITH 13 FIGURES)

This paper is a monograph of the species of *Puccinia* known to occur on *Salvia* in South America. A previous monograph by Baxter and Cummins (**2**) reviewed the North American members of this group of rusts. In the present paper eleven South American species of *Puccinia* on *Salvia* are redescribed and redefined. In addition, two species are described as new.

Knowledge of the South American *Salvia* rusts is based chiefly on the Holway collections and to a lesser extent on specimens collected by Mayor, Lagerheim, and others. The Sydows (**8**), in 1904, published descriptions and illustrations of the species known at that time. Two new species were described from Colombia by Mayor (**7**) in 1913, and in 1932 seven of the species treated in the present paper were described as new by Jackson and Holway (**5**).

The urediospores of these rusts, as in the North American members of the group, have zonate, equatorial or subequatorial germ pores, except in *Puccinia roestelliformis* Lagerh., which is distinct in having urediospores with scattered pores. With respect to teliospore characters the South American species of *Puccinia* on *Salvia* can be divided into two groups. In one group the teliospores germinate only after a resting period and the teliospore wall is sculp-

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tured throughout, with the marking ranging from striate or finely punctate-verrucose to a coarsely verrucose or rugose type of sculpturing. The other group is characterized by teliospores which germinate without a resting period and in which the wall is thinner, paler, and, except in one species, smooth throughout.

In separating species or groups of species in the key, teliospore characters have been used almost exclusively. The most reliable of these are: (a) the time of germination, (b) the nature of the spore wall, (c) the persistence and length of the pedicel, and (d) the position of the pore in the lower cell. The only use of an urediospore character has been in separating two species on the basis of the thickness of the urediospore wall.

KEY AND DESCRIPTION OF SPECIES

Teliospores germinating without a resting period; wall smooth throughout, or smooth in the lower half and roughened above.
- Teliospore pedicel 12–23 μ wide, 160–300 μ long.
  - Teliospores 20–30 × 50–90 μ.....1. *P. astricta.*
  - Teliospores 16–23 × 100–155 μ.....2. *P. roestelliformis.*
- Teliospore pedicel less than 12 μ wide, less than 160 μ long.
  - Teliospores 43–66 μ long.
    - Teliospores 16–23 μ wide; apical umbo smooth.....3. *P. albiccra.*
    - Teliospores 20–30 μ wide; apical umbo striate.....4. *P. soledadensis.*
  - Teliospores 26–43 μ long.....5. *P. conspersa.*

Teliospores germinating after a resting period; wall striate, verrucose, or rugose throughout.
- Teliospore wall 5–8 μ thick; pedicel persistent, 100–150 μ long.
  - Pore of lower cell near the pedicel.....6. *P. gilliesii.*
  - Pore of lower cell next to the septum.
    - Urediospore wall 1–1.5 μ thick.....7. *P. aenigmatica.*
    - Urediospore wall 3 μ thick.....8. *P. porphyretica.*
- Teliospore wall less than 5 μ thick; pedicel fragile, less than 50 μ long.
  - Teliospore wall rugose, dark chestnut-brown.
    - Pore of lower cell next to the septum.....9. *P. conturbata.*
    - Pore of lower cell next to the pedicel.....10. *P. mitoensis.*
  - Teliospore wall striate or finely verrucose, light chestnut or cinnamon-brown.
    - Pore of lower cell next to the septum.....11. *P. sana.*
    - Pore of lower cell next to the pedicel.
      - Teliospores 28–37 μ long.....12. *P. sphacelicola.*
      - Teliospores 36–43 μ long.....13. *P. striatifera.*
- Teliospore wall less than 5 μ thick; pedicel persistent, 50–150 μ long.
  - Pedicel thick-walled, 100–150 μ long.....14. *P. impedita.*
  - Pedicel thin-walled, 50–80 μ long.....15. *P. farinacea.*

1. PUCCINIA ASTRICTA Jacks & Holw. Mycologia **24**: 74. 1932.

Spermagonia epiphyllous, in groups, globoid, cinnamon-brown, 100–150 μ in diameter. Aecia and uredia lacking. Telia amphigenous, chiefly hypophyllous, in groups accompanying the spermagonia, round, compact, cinnamon-brown, becoming cinereous from germination, 0.3–1.0 mm. in diameter; teliospores narrowly ellipsoid, subclavate, or broadly fusiform, 20–30 × 50–90 μ, rounded or somewhat narrowed at both ends, constricted at the septum, germinating without a resting period; wall pale cinnamon or yellowish, smooth, 1–2 μ in thickness, thickened apically to 3–4 μ; pore of upper cell apical, of lower next to the septum; pedicel hyaline, thin-walled, persistent, 12–23 μ in width, 160–200(–250) μ long.

HOSTS AND DISTRIBUTION: *Salvia* sp., Ecuador.

TYPE LOCALITY: Huigra, Ecuador, on *Salvia* sp.

ILLUSTRATIONS: FIG. 1 (from type).

SPECIMENS EXAMINED: On *Salvia* sp.: Huigra, Ecuador, Aug. 4, 1920, Holway *812 A* (type).

Puccinia astricta, known only from the type locality, is distinct from the other rusts treated herein, being the only lepto-species in the group. The long, stout teliospore pedicels are characteristic of *P. astricta* and *P. roestelliformis*.

2. PUCCINIA ROESTELIIFORMIS Lagerh. apud Sydow, Monogr. Ured. **1**: 292. 1904.

Spermagonia epiphyllous, in groups, subepidermal, globoid, cinnamon-brown or yellowish, 130–170 μ in diameter. Aecia hypophyllous, roestelioid, 0.3–1.0 mm. in diameter, 1–4 mm. high, scattered or in groups up to 5 mm. across; peridial cells angularly elliptical, hexagonal or rhomboidal, hyaline, 24–26 × 32–48 μ in surface view, inner wall 6–10 μ, coarsely verrucose, outer wall 3–6 μ, striate; aeciospores broadly ellipsoid, globoid, or pyriform, 23–30 × 30–43 μ; wall pale yellow, 3–5(–7) μ in thickness, tessellate. Uredia hypophyllous, scattered, round, compact, paraphysate, pale cinnamon-brown, 0.2–1.0 mm. in diameter; urediospores mostly globoid, 20–28 × 20–28 μ, occasionally broadly ellipsoid, 20–25 × 23–30 μ; wall pale cinnamon or yellowish, closely and finely punctate-verrucose, 1.5–2.5 μ thick, pores 6–9, scattered, located in slightly concave areas of the wall, each covered by a conspicuous hyaline papilla 1–2 μ thick; uredial paraphyses hyaline, thin-walled, cylindrical or clavate, 13–26 μ broad at the apex, up to 135 μ long. Telia hypophyllous, scattered, round, compact, becoming gelatinous when moist, cinnamon-brown, 0.2–1.5 mm. in diameter; teliospores cylindrical, (13–)16–23(–27) × (80–)100–155(–170) μ, rounded

above, obtuse or slightly rounded below, slightly or not constricted at the septum, germinating without a resting period; wall cinnamon-brown, paler over the pores, smooth, 1–2 μ, thickened to 3–4 μ at the angles of the septum and at the apex; pore of upper cell apical, of lower next to the septum; pedicel hyaline, thin-walled, persistent, 13–23 μ in width, 250–300(–330) μ long.

HOSTS AND DISTRIBUTION: *Salvia corrugata* Vahl, Ecuador; *Salvia* sp., Ecuador.

TYPE LOCALITY: Guayras-caja, Ecuador, on *Salvia* sp.

ILLUSTRATIONS: Sydow, Monogr. Ured. **1**: Tab. XIX, FIG. *275*; this paper, FIG. *2* (from type).

EXSICCATI: Reliq. Holw. *129*.

SPECIMENS EXAMINED: On *Salvia corrugata:* Biblian, Prov. Canar, Ecuador, Sept. 9, 1920, Holway *966:* Ambato, Dept. Tunguragua, Ecuador, March, 1919, Pachano *192;* Guaranda, Prov. Bolivar, Ecuador, April *26*, 1942, Haught *3281*. On *Salvia* sp.: Guayras-caja, Prov. Chimborazo, Ecuador, Sept., 1891, G. Lagerheim (type).

This species differs strikingly from other *Salvia* rusts in several respects, the chief points of difference being the long, cylindrical teliospores, the tremelloid nature of the telia, and the presence of paraphyses in the uredia. The urediospores are distinct from those of the other South American species in having 6–9 scattered pores. In this and in shape and wall characters the urediospores of *P. roesteliiformis* show a marked resemblance to those of *P. bithynica* Magn., a species occurring on *Salvia* in the Mediterranean region.

The telia of *P. roesteliiformis* become somewhat gelatinous when moist, resembling those of species of *Gymnosporangium* in this respect, as Sydow (**8**) has pointed out. The roestelioid nature of the aecia, upon which the species name is based, is another point of similarity. However, the host relationship of *P. roesteliiformis* and the presence of paraphysate uredia in this species would seem to leave little doubt that Lagerheim was correct in naming it as a species of *Puccinia* rather than placing it in *Gymnosporangium*.

The aecia and aeciospores of *P. roesteliiformis* are much like those of *P. gilliesii* Speg. and *P. sphacelicola* Jacks. & Holw.; the aeciospores differ, however, in lacking the papilla or beak characteristic of the latter two species. Jackson (**5**) has described the

aeciospore wall in *P. roestelliformis* as "reticulate"; actually it is tessellate, with large, flat, closely-fitting tubercles.

3. PUCCINIA ALBICERA Jacks. & Holw. Mycologia **24**: 73. 1932.

Spermagonia epiphyllous, in groups, subepidermal, globoid, chestnut-brown, 100–180 μ in diameter. Aecia amphigenous, chiefly epiphyllous, aecidioid, in groups up to 2 mm. across; peridial cells oblong or elliptical, hyaline, 16–30 × 30–40 μ in surface view, inner wall 2–3 μ, punctate-verrucose, outer wall 10–13 μ, finely striate; aeciospores globoid, ellipsoid, or oblong, 16–26 × 16–33 (–43) μ, wall yellowish, 1–2 μ thick, coarsely verrucose, with square, flat tubercles. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown or yellowish, 0.1–0.5 mm. in diameter; urediospores oblate-spheroid, 20–25 μ broad by 17–23 μ high; wall cinnamon-brown, echinulate, 1–1.5 μ thick, pores 1 or 2, about ½ subequatorial. Telia hypophyllous, scattered, round, compact, cinnamon- or chestnut-brown, becoming cinereous from germination, 0.2–1.0 mm. in diameter; teliospores clavate, cylindrical or narrowly ellipsoid (13–)16–23 × (43–)50–56(–66) μ, rounded or truncate above, somewhat narrowed below, sharply constricted at the septum, germinating without a resting period; wall cinnamon-brown, smooth, 1–1.5 μ thick, pore of upper cell apical, of lower at the septum, each covered by a hyaline umbo 3–6 μ thick; pedicel hyaline thin-walled, fragile, frequently breaking away completely, occasionally persisting and up to 80 μ long.

HOSTS AND DISTRIBUTION: *Salvia derasa* Benth., Ecuador; *Salvia tortuosa* Kunth, Ecuador.

TYPE LOCALITY: Quito, Ecuador, on *Salvia derasa*.

ILLUSTRATIONS: FIG. 3 (from type).

SPECIMENS EXAMINED: On *Salvia derasa:* Quito, Ecuador, Aug. 14, 1920, Holway *889* (type). On *Salvia tortuosa:* Quito, Ecuador, Aug. 21, 1920, Holway *930;* Pichincha, Ecuador, Dec. 29, 1938, Balls *5819*.

Puccinia albicera shows some resemblance to *P. soledadensis* Mayor, but differs from that species in having narrower teliospores and smaller urediospores. An additional point of difference in teliospore characteristics is in the nature of the apical umbo, which is smooth in *P. albicera*, but striate in *P. soledadensis*.

4. PUCCINIA SOLEDADENSIS Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 494. 1913.

Uredo archeriana Kern, Thurston & Whetzel, Mycologia **25**: 485. 1933.

Spermagonia and aecia unknown. Uredia hypophyllous, scattered, round, pulverulent, chestnut-brown, 0.2–1.0 mm. in diameter; urediospores variable, oblate-spheroid and 23–28 μ broad by 20–25 μ high, obovoid, 20–25 × 21–33 μ, or ellipsoid, 20–28 × 25–40(–43) μ; wall usually dark cinnamon-brown, occasionally paler, sparsely and prominently echinulate, with conspicuous conical spines, 1.5–2 μ in thickness, pores 2, rarely 3, equatorial or ⅓–½ subequatorial. Telia hypophyllous, scattered, round, compact, pulvinate, chestnut-brown, 0.3–1.5 mm. in diameter; teliospores narrowly ellipsoid, oblong, or subclavate, 20–30 × 43–56 μ, rounded above, rounded or narrowed below, constricted at the septum, germinating without a resting period; wall cinnamon-brown, smooth, 1–1.5 μ thick; pore of upper cell apical, capped by a striate, hyaline or yellowish umbo 4–7 μ thick, pore of lower cell next to the septum, covered by a hyaline or yellowish umbo 3–4 μ thick; pedicel hyaline, thin-walled, persistent, usually equal to the spore in length, occasionally up to 70 μ long.

HOSTS AND DISTRIBUTION: *Salvia angulata* Benth., Venezuela; *Salvia bogotensis* Benth., Colombia; *Salvia calocalicina* Briq., Colombia; *Salvia latens* Benth., Colombia; *Salvia* sp., Colombia.

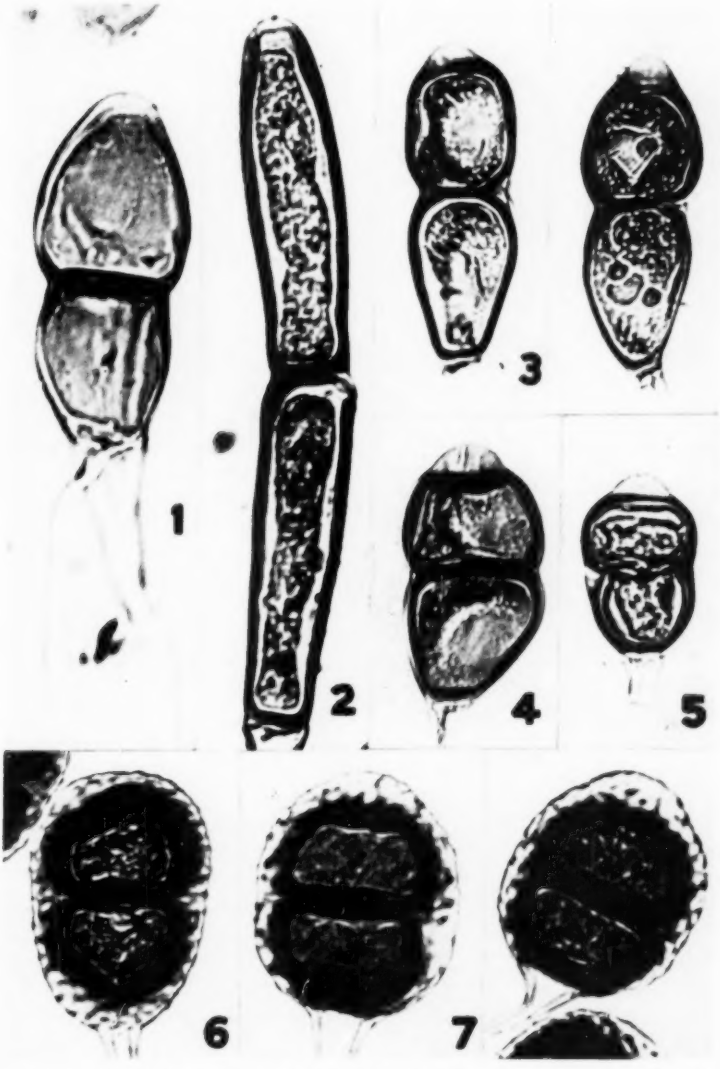
TYPE LOCALITY: Soledad, Colombia, on *Salvia* sp.

ILLUSTRATIONS: Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: FIG. 26; this paper, FIG. 4 (from type).

SPECIMENS EXAMINED: On *Salvia angulata:* Merida, Venezuela, Jan. 20, 1928, Pittier *12642*. On *Salvia bogotensis:* Soata, Dept. Boyaca, Colombia, Sept. 6, 1938, Cuatrecasas *1006*. On *Salvia calocalicina:* Salento, Dept. Caldas, Colombia, July 25–31, 1922, Pennell *8901*. On *Salvia latens:* Sta. Elena, Colombia, Dec. 28, 1930, Archer *H-201* (type of *Uredo archeriana*). On *Salvia* sp.: Soledad, Colombia, Oct. 6, 1910, Mayor *79* (type).

Mayor (**7**) described this species from a specimen having only the telial stage, and pointed out that its development resembled that of a lepto-form. However, during the course of the present study the writer obtained specimens of *P. soledadensis* in which uredia as well as telia were found. These specimens are on leaves of *Salvia bogotensis* and *S. calocalicina* obtained from the phanerogamic collections of the U. S. National Herbarium.

The urediospores of *P. soledadensis* are variable in shape, ranging from oblate-spheroid to narrowly ellipsoid. The wall is characteristically dark cinnamon, with large, conical spines.



FIGS. 1–8. 1. Teliospore of *Puccinia astricta* (from type), ×800. 2. Teliospore of *P. roesteliiformis* (from type), ×800. 3. Teliospores of *P. albicera* (from type), ×800. 4. Teliospore of *P. soledadensis* (from type), ×800. 5. Teliospore of *P. conspersa* (from type), ×800. 6. Teliospore of *P. gilliesii* (from type), ×800. 7. Teliospores of *P. aenigmatica* (from type), ×800.

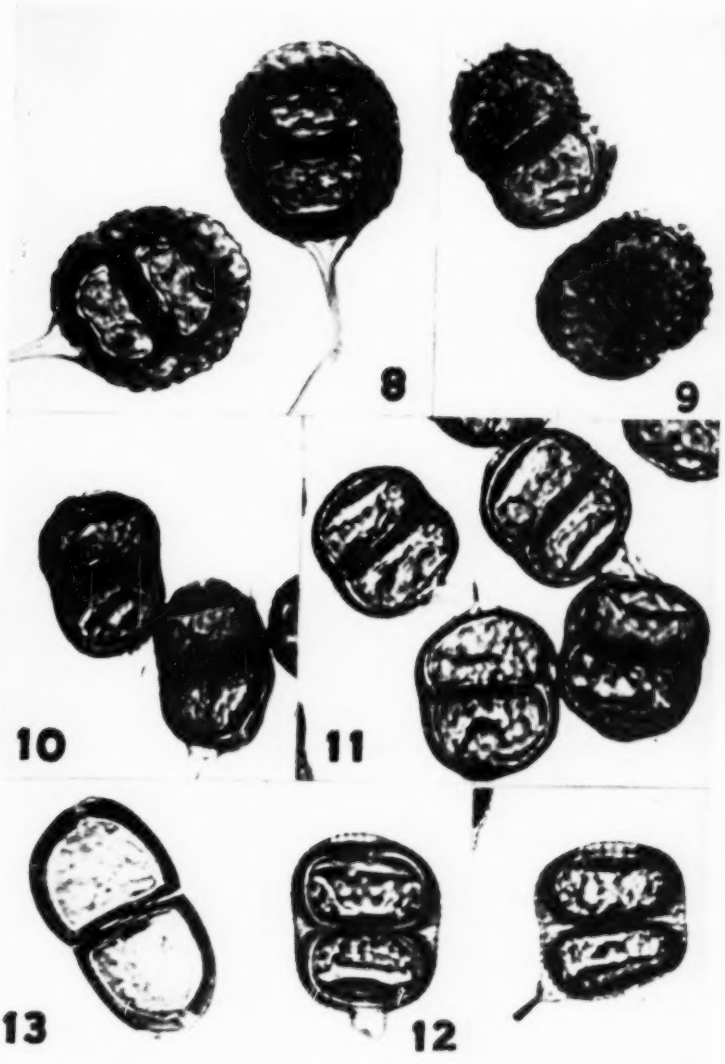
An examination of the type specimen of *Uredo archeriana* during this study revealed the presence of a sorus bearing teliospores of *P. soledadensis*. The urediospores in this specimen are larger and more narrowly ellipsoid than those of the other specimens of *P. soledadensis*, but agree well in wall color and type of echinulation. In accord with these findings *Uredo archeriana* is reduced to synonymy with *P. soledadensis*.

5. PUCCINIA CONSPERSA Diet. Hedwigia **36**: 30. 1897.

Puccinia uliginosa Speg. Anal. Mus. Nac. Hist. Nat. Buenos Aires **23**: 26. 1912 (not *P. uliginosa* Juel 1894).

Spermagonia epiphyllous, in groups, subepidermal, globoid, cinnamon-brown, 85–165 μ in diameter. Aecia amphigenous, aecidioid, scattered or in groups up to 2 mm. across or up to 3 mm. in length along the veins; peridial cells elliptical or oblong, hyaline, 20–27 × 27–43 μ in surface view, inner wall 2–4 μ, coarsely verrucose, the tubercles forming conspicuous ridges, outer wall 6–10 μ, finely verrucose; aeciospores globoid or ellipsoid, occasionally angularly ellipsoid or oblong, 16–23 × 23–33 μ; wall pale yellow, 1–2 μ thick, finely verrucose. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown, orange, or yellowish, 0.1–0.7 mm. in diameter; urediospores asymmetrical, broadly ellipsoid or obovoid with the pores in surface view, narrower and more nearly oblong with the pores in optical section, 16–23 × 20–26(–30) μ; wall pale cinnamon, echinulate, about 1 μ thick, pores 2, equatorial. Telia hypophyllous, occasionally also caulicolous, scattered or in groups up to 2 mm. across, round or elliptic, pulvinate, somewhat compact, chestnut-brown, 0.1–0.5 mm. in diameter on leaves, 0.5–1.5 mm. across and up to 3 mm. long on stems; teliospores ellipsoid or oblong, 17–23(–28) × 26–40(–43) μ, rounded at both ends, not or slightly constricted at the septum, germinating without a resting period; wall cinnamon-brown or yellowish, smooth below, verruculose or nearly smooth above, 1–1.5 μ thick, thickened to 2–3 μ at the angles of the septum, pore of upper cell apical, of lower next to the septum, each capped by a hyaline umbo 2–4 μ thick; pedicel hyaline, thin-walled, fragile, frequently breaking away completely, when present about ½ the length of the spore, occasionally up to 65 μ long.

HOSTS AND DISTRIBUTION: *Salvia arenaria* St. Hil., Brazil; *Salvia procurrens* Benth., Argentina; *Salvia sellowiana* Benth., Brazil; *Salvia serrata* Benth. (*Salvia pallida* Benth.), Argentina; *Salvia splendens* Sellow, Brazil, Argentina; *Salvia uliginosa* Benth., Argentina; *Salvia* sp., Brazil.



FIGS. 8–13. 8. Teliospores of *P. porphyretica* (from type), ×800. 9. Teliospores of *P. conturbata* (from type), ×800. 10. Teliospores of *P. mitoensis* (from type), ×800. 11. Teliospores of *P. sana* (from type), ×800. 12. Teliospores of *P. sphacelicola* (from type), ×800. 13. Teliospore of *P. striatifera* (from type), ×800.

TYPE LOCALITY: Serra Geral, Brazil, on *Salvia* sp.

ILLUSTRATIONS: Sydow, Monogr. Ured. **1**: Tab. XIX, FIG. *273*; this paper, FIG. 5 (from type).

EXSICCATI: Reliq. Holw. *431, 433, 439.*

SPECIMENS EXAMINED: On *Salvia arenaria:* São Paulo, Brazil, May 2, 1922, Holway *1802.* On *Salvia procurrens:* La Plata, Argentina, March 28, 1947, Lindquist. On *Salvia sellowiana:* São Paulo, Brazil, May 6, 1922, Holway *1806.* On *Salvia serrata:* La Plata, Argentina Dec. 3, 1921, Lindquist. On *Salvia splendens:* Rio de Janeiro, Brazil, Aug. 15, 1921, Holway *1039;* La Plata, Argentina, April 17, 1924, Marchionatto *8352.* On *Salvia uliginosa:* Buenos Aires, Argentina, June 4, 1910, Buisel (type of *P. uliginosa* Speg.); La Plata, Argentina, April 12, 1947, Lindquist. On *Salvia* sp: Serra Geral, Brazil, April, 1891, Ule *1776* (type); Rio De Janeiro, Brazil, Nov. 8, 1921, Holway *1285, 1300;* São Paulo, Brazil, July 2, 1922, Holway *1993.*

The teliospores of *Puccinia conspersa* are somewhat like those of *P. soledadensis* in general appearance, but are much smaller. The urediospores are characteristically asymmetrical with the pores located in the flattened sides.

Examination of the type specimen of *P. uliginosa* Speg. shows that Jackson (**5**) was correct in listing this species as a synonym of *P. conspersa.*

PUCCINIA CONSPERSA var. **paramensis** stat. nov.

Puccinia paramensis Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 493. 1913.

A forma typica speciei differt teliosporis 16–22 × 22–30 μ; membrana uniformiter 1 μ crassa, ad apicem granulo-verrucosa.

Spermagonia and aecia unknown. Uredia, urediospores, and telia as in the typical form of *P. conspersa.* Teliospores broadly ellipsoid, 16–22 × 22–30 μ, rounded at both ends, slightly constricted at the septum, germinating without a resting period; wall about 1 μ thick, pale cinnamon or yellowish, granular-verrucose above, appearing smooth in the lower half, pore of upper cell apical, of lower next to the septum, each with a hyaline inconspicuous umbo 1–2.5 μ thick; pedicel hyaline, thin-walled, fragile, usually breaking away at the point of attachment.

On leaves of *Salvia carnea* Kunth in Colombia and on leaves of *Salvia killipiana* Epl. in Venezuela.

HOSTS AND DISTRIBUTION: *Salvia carnea* Kunth, Colombia; *Salvia killipiana* Epl., Venezuela.

TYPE LOCALITY: Paramos, Colombia, on *Salvia carnea*.

ILLUSTRATIONS: Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: FIG. 25.

SPECIMENS EXAMINED: On *Salvia carnea:* Paramos, Colombia, Oct. 5, 1910, Mayor *83* (type). On *Salvia killipiana:* Lagua Negra, Merida, Venezuela, Feb. *27*, 1931, Reed *839*.

In establishing *Puccinia paramensis,* Mayor (**7**) described only the telial stage. A close examination of his specimen during this study disclosed the presence of a few uredia bearing urediospores which agree well with those of *P. conspersa.* Later a specimen on *Salvia killipiana,* agreeing with Mayor's species and having abundant uredia and telia, was obtained from the phanerogamic collections of the U. S. National Herbarium. The teliospores in this specimen, although smaller and thinner-walled than those of *P. conspersa,* resemble the latter closely in the color and marking of the wall. It seems advisable on the basis of these observations to reduce *P. paramensis* to varietal rank.

6. PUCCINIA GILLIESII Speg. Anal. Soc. Ci. Argent. **47**: 265. 1899.

Puccinia obesa Sydow, Monogr. Ured. **1**: 289, 1904.

Spermagonia not seen. Aecia hypophyllous and caulicolous, short-cylindric, in groups up to 3 mm. across on leaves, up to 12 mm. long and causing hypertrophy and distortion on stems; peridial cells elliptical, angularly oval or oblong, pale yellow, 16–33 × 43–100 μ in surface view, inner wall 7–10 μ thick, verrucose, outer wall 5–9 μ thick, finely striate; aeciospores variable in shape, ellipsoid, pyriform, broadly fusiform, or oblong, 12–21 × 28–58 μ, wall pale yellow, verrucose-tesselate with large, flat tubercles, 3–4 μ thick at the sides, thickened to 5–7 μ at the ends, frequently prolonged at one end to form a conspicuous beak 7–14(–18) μ long. Uredia amphigenous, scattered, roundish, pulvinate, compact, light chestnut or cinnamon-brown, 0.5–4.0 mm. in diameter; urediospores broadly ellipsoid, obovoid or globoid, 23–30 × 27–37 μ; wall cinnamon-brown or yellowish, 4–6 μ thick, sparsely and prominently echinulate, with large conical spines, pores 3–4, equatorial, obscure. Telia amphigenous, scattered, roundish, pulverulent, blackish brown, 0.5–2.0 mm. in diameter; teliospores ellipsoid, 27–39 × 37–52 μ, rounded at both ends, not or slightly constricted

at the septum; wall 5–8 μ thick, thickened to 10–12 μ over the pores, obscurely and evenly verrucose with low, flat tubercles, laminate, the inner layer chocolate- or dark chestnut-brown, the outer cinnamon or yellowish, pore of upper cell apical, of lower about $\frac{2}{3}$ of the distance between the septum and the pedicel, each pore covered by a yellowish, slightly umbonate thickening; pedicel brownish or yellow next to the spore, becoming hyaline downward, thick-walled, persistent, occasionally attached obliquely, 6–8 μ in width at the point of attachment, up to 150 μ long.

HOSTS AND DISTRIBUTION: *Salvia bangii* Rusby, Bolivia; *Salvia gilliesii* Benth., Argentina; *Salvia humboldtiana* Dietr., Ecuador.

TYPE LOCALITY: Cordoba, Argentina, on *Salvia gilliesii*.

ILLUSTRATIONS: Sydow, Monogr. Ured. **1**: Tab. XIX, FIG. *272*; this paper, FIG. 6 (from type).

SPECIMENS EXAMINED: On *Salvia bangii:* Cochabamba, Bolivia, Feb. 23, 1920, Holway *314*. On *Salvia gilliesii:* La Falda de la Punilla, Prov. Cordoba, Argentina, Sept. 12, 1896, Hieronymus (type of *P. obesa*); Cordoba, Argentina, May, 1899, Stuckert *6954* (type); Cacheuta, Argentina, March 9, 1909, Spegazzini: Prov. Salta, Argentina, April 8, 27, 1925, Venturi *3674, 3764;* San Rafael, Mendoza, Argentina, Nov. 30, 1940, Leal *6916;* Las Heras, Mendoza, Argentina, July 27, 1941, Leal *7645;* Las Heras, Mendoza, Argentina, May 7, 1944, Leal *9332*. On *Salvia humboldtiana:* Ambato, Prov. Tunguragua, Ecuador, July 9, 1939, Balls *7152*.

Puccinia gilliesii is readily distinguishable from other species on *Salvia* because of the thick-walled, prominently echinulate urediospores. The number of germ pores is somewhat variable, with 3- and 4-pored urediospores occurring in approximately equal numbers. The aecia and aeciospores of this species are much like those of *P. rocs'eliiformis* and *P. sphaceticola*. The aeciospore wall is distinctive, however, in being markedly thickened at the ends and frequently prolonged to form a conspicuous beak.

Although the teliospores of *P. gilliesii* resemble those of *P. aenigmatica* Jacks. & Holw. and *P. porphyretica* Jacks. & Holw. in having thick, bilaminate walls, they differ in the position of the pore in the lower cell and in the type of wall sculpturing.

7. PUCCINIA AENIGMATICA Jacks. & Holw. Mycologia **24**: 72. 1932.

Spermagonia and aecia unknown. Uredia amphigenous, scattered, round, pulverulent, chestnut-brown, 0.2–0.5 mm. in diameter; urediospores oblate-spheroid, 23–30 μ broad by 20–23 μ high; wall dark cinnamon-brown, echinulate, 1–1.5 μ thick, pores 2, rarely 3 or 4, usually near the hilum, occasionally ⅓–½ subequatorial. Telia amphigenous, scattered, round, pulverulent, blackish brown, 0.3–1.0 mm. in diameter; teliospores broadly ellipsoid or globoid, 30–38 × 33–46 μ, rounded at both ends, not constricted at the septum; wall laminate, chestnut-brown, coarsely verrucose, 5–8 μ thick, thickened to 10–14 μ and paler over the pores, pore of upper cell apical, of lower near the septum; pedicel hyaline, thick-walled, persistent, 8–11 μ wide at the point of attachment, up to 150 μ long.

HOSTS AND DISTRIBUTION: *Salvia bangii* Rusby (*Salvia erythradena* Briq.), Bolivia; *Salvia haenkei* Benth., Bolivia; *Salvia orbignaei* Benth. (*Salvia kuntzeana* Briq.), Bolivia; *Salvia sarmentosa* Epl., Peru.

TYPE LOCALITY: Cochabamba, Bolivia, on *Salvia orbignaei* (*Salvia kuntzeana*).

ILLUSTRATIONS: FIG. 7 (from type).

EXSICCATI: Reliq. Holw. *417, 418* (the latter as *Puccinia mitrata* Syd.).

SPECIMENS EXAMINED: On *Salvia bangii:* Cochabamba, Bolivia, March 10, 1920, Holway *386*. On *Salvia haenkei:* Cochabamba, Bolivia, June, 1947, Cardenas *225*. On *Salvia orbignaei: Cochabamba*, Bolivia, March 1, 1920, Holway *358* (type). On *Salvia sarmentosa:* Ollantaytambo, Peru, April 26, 1915, Cook & Gilbert *375*.

One of the specimens cited above, Cardenas' No. *225* on *S. haenkei*, has urediospores which range in shape from globoid to broadly ellipsoid and thus do not agree completely with those of the type. However, the position of the pores and the thickness of the wall, as well as the size of uredia, indicate that this specimen should be referred to *P. aenigmatica*.

Holway's specimen on *S. bangii* (*S. erythradena*) was originally determined as *P. mitrata* by Jackson (**5**). Although the urediospores in this specimen approach those of *P. mitrata* in size, in all

other respects the specimen agrees well with *P. aenigmatica* and is accordingly referred to that species.

8. PUCCINIA PORPHYRETICA Jacks. & Holw. Mycologia **24**: *76*, 1932.

Spermagonia and aecia unknown. Uredia amphigenous, scattered, round, pulverulent, chestnut-brown, 0.4–3.0 mm. in diameter; urediospores broadly ellipsoid or obovoid, 20–26 × 24–34 μ; wall cinnamon-brown, prominently echinulate, about 3 μ thick, pores 2, equatorial. Telia amphigenous, scattered, round, pulverulent; chocolate-brown, 0.3–5.0 mm. in diameter; teliospores broadly ellipsoid or globoid, 26–38 × 30–46 μ, rounded at both ends, not constricted at the septum; wall distinctly laminate, uniformly light chestnut- or cinnamon-brown, occasionally dark chestnut-brown, coarsely verrucose, 5–8 μ thick, thickened to 9–14 μ and paler over the pores, pore of upper cell apical, of lower next to the septum; pedicel hyaline, thick-walled, persistent, 6–10 μ in width at the point of attachment, up to 130 μ long.

HOSTS AND DISTRIBUTION: *Salvia haenkei* Benth. (*Salvia bridgesii* Britton, *Salvia pseudoaviculariis* Briq.), Bolivia; *Salvia* sp., Peru.

TYPE LOCALITY: Cuzco, Peru, on *Salvia* sp.

ILLUSTRATIONS: FIG. 8 (from type).

EXSICCATI: Reliq. Holw. *416, 419, 425.*

SPECIMENS EXAMINED: On *Salvia haenkei:* Cochabamba, Bolivia, Feb. 26, 1920, Holway *329;* Sorata, Bolivia, April 15, 1920, Holway *527.* On *Salvia* sp.: Cuzco, Peru, July 4, 1920, Holway *761* (type).

Puccinia porphyretica and *Puccinia aenigmatica* are representatives of a group of New World *Salvia* rusts which resemble one another closely with respect to teliospore characters. The other species in this group are *Puccinia mitrata* Syd. and *Puccinia gentilis Arth.*, known to occur only in North America (2). All have broad teliospores with thick, coarsely verrucose, bilaminate walls. These rusts can be separated on the basis of urediospore characters, size of uredia, and the nature of the teliospore pedicel. The chief points of distinction are given in the following key:

Teliospore pedicel thin-walled, 70–100 μ long.
Urediospores 16–21 × 20–23 μ.....*P. mitrata.*
Urediospores 20–23 × 23–29 μ.....*P. gentilis.*

Teliospore pedicel thick-walled, 100–150 μ long.
Urediospore wall 1–1.5 μ thick, uredia 0.2–0.5 mm. in diameter ..... *P. aenigmatica.*
Urediospore wall 3 μ thick, uredia 0.4–3.0 mm. in diameter ..... *P. porphyretica.*

9. PUCCINIA CONTURBATA Jacks. & Holw. Mycologia **24**: 75. 1932.

Spermagonia and aecia unknown. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown, 0.2–0.5 mm. in diameter; urediospores oblate-spheroid, 23–26 μ broad by 20–22 μ high, or obovoid, 18–24 × 22–25 μ; wall cinnamon-brown, closely echinulate, 1–1.5 μ thick in the upper two-thirds, becoming 2–2.5(–3) μ thick and darker in the lower third, pores 2, occasionally 3, about ½ subequatorial. Telia hypophyllous, scattered, round, pulverulent, blackish brown, 0.1–0.5 mm. in diameter; teliospores ellipsoid or oblong, 23–26 × 30–36(–40) μ, rounded at both ends, constricted at the septum; wall dark chestnut-brown, rugose-reticulate, 2–3 μ in thickness, thickened to 4–6 μ and paler at the apex, pore of upper cell apical, of lower next to the septum; pedical hyaline, thin-walled, fragile, usually breaking away at the point of attachment.

HOSTS AND DISTRIBUTION: *Salvia quitensis* Benth., Ecuador; *Salvia* sp., Ecuador.

TYPE LOCALITY: Huigra, Ecuador, on *Salvia* sp.

ILLUSTRATIONS: FIG. 9 (from type).

SPECIMENS EXAMINED: On *Salvia quitensis:* Cuenca, Ecuador, Sept. 15, 1920, Holway *992*. On *Salvia* sp.: Huigra, Ecuador, Aug. 4, 1920, Holway *842* (type).

The teliospores of this species show some resemblance to those of a North American species, *Puccinia diutina* Mains & Holw. The chief point of distinction between the two species is in the nature of the urediospore wall. In *P. conturbata* the wall is thin in the upper two-thirds, but markedly thickened basally, while the condition in *P. diutina* is the reverse of this. An additional point of difference is in the position of the urediospore pores, which in *P. diutina* are basal next to the hilum.

10. **Puccinia mitoensis** sp. nov.

Spermagoniis et aeciis adhuc ignotis. Urediis hypophyllis, sparsis, rotundatis, pulverulentis, cinnamomeo-brunneis, 0.2–0.5 mm. diam.; urediosporis late globoideis, 23–30 μ latis, 20–26 μ altis; membrana cinnamomeo-brunnea, moderate echinulata, 1.5–2 μ crassa, poris germ. 2 vel 3, subaequatorialibus vel aequatoriaibus. Teliis hypophyllis, sparsis, rotundatis, pulverulentis,

atro-brunneis, 0.1–0.5 mm. diam.; teliosporis ellipsoideis vel oblongis, 18–24 × 26–32 μ, utrinque rotundatis, medio non vel leniter constrictis; membrana castaneo-brunnea, 1.5–2.5 μ crassa, supra poros usque ad 4 μ in umbonem pallidiorem incrassata, obscure rugoso-reticulata; poro superiore apicali, inferiore juxta pedicellum sito; pedicello hyalino, fragili, brevissimo.

Spermagonia and aecia unknown. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown, 0.2–0.5 mm. in diameter; urediospores oblate-spheroid, 23–30 μ broad by 20–26 μ high; wall cinnamon-brown, moderately echinulate, 1.5–2 μ thick, pores 2, occasionally 3, about ½ subequatorial or occasionally equatorial. Telia hypophyllous, scattered, round, pulverulent, blackish brown, 0.1–0.5 mm. in diameter; teliospores ellipsoid or oblong, 18–24 × 26–30 μ, rounded at both ends, not or slightly constricted at the septum; wall chestnut-brown, obscurely rugose-reticulate, 1.5–2.5 μ in thickness, thickened to 3–4 μ at the angles of the septum, pore of upper cell apical, of lower next to the pedicel, each capped by a flat yellowish umbo about 2 μ thick; pedicel hyaline, thin-walled, fragile, usually breaking away at the point of attachment.

On leaves of *Salvia punctata* var. *glabra* in Peru.

HOSTS AND DISTRIBUTION: *Salvia punctata* R. & P. var. *glabra* Epl., Peru.

TYPE LOCALITY: Mito, Peru, on *Salvia punctata* var. *glabra*.

ILLUSTRATIONS: FIG. 10 (from type).

SPECIMEN EXAMINED: On *Salvia punctata* var. *glabra:* Mito, Peru, July 8, 22, 1922, Macbride & Featherstone.

Puccinia mitoensis is much like *P. conturbata*, but differs in having smaller, thinner-walled teliospores in which the pore of the lower cell is located next to the pedicel. The finer, less conspicuous marking of the teliospore wall in *P. mitoensis* serves further in distinguishing the two species.

11. PUCCINIA SANA Jacks. & Holw. Mycologia **24**: 77. 1932.

Spermagonia and aecia present but too old for accurate description. Uredia hypophyllous, scattered, round, pulverulent, cinnamon-brown, 0.1–0.5 mm. in diameter; urediospores oblate-spheroid, 23–26 μ broad by 20–23 μ high, or occasionally obovoid or ellipsoid, 20–23 × 23–26 μ; wall connamon-brown, moderately echinulate, 1–1.5 μ thick, pore 1, equatorial. Telia hypophyllous, scattered, round, pulverulent, chestnut- or chocolate-brown, 0.1–0.5 mm. in diameter; teliospores broadly ellipsoid or oblong, 20–27 × 26–36 μ, rounded at both ends, not or slightly constricted at the septum; wall light chestnut-brown or cinnamon-brown, finely and evenly

verrucose, 1.5–2 μ thick, thickened to 3 μ at the angles of the septum, pore of upper cell apical, of lower next to the septum, each capped by an inconspicuous hyaline umbo 1–2.5 μ thick; pedicel hyaline, thin-walled, fragile, usually broken off at or near the spore, occasionally up to 40 μ long.

HOSTS AND DISTRIBUTION: *Salvia leucocephala* Kunth, Ecuador; *Salvia* sp., Ecuador.

TYPE LOCALITY: Huigra, Ecuador, on *Salvia leucocephala*.

ILLUSTRATIONS: FIG. 11 (from type).

SPECIMENS EXAMINED: On *Salvia leucocephala* Kunth: Huigra, Ecuador, Aug. 4, 1920, Holway *835* (type); Huigra, Ecuador, Aug. 4, 1920, Holway *839*. On *Salvia* sp.: Chimborazo, Ecuador, Sept., 1891, Lagerheim.

Jackson (**5**) pointed out that *Puccinia sana* appeared to be much the same as an undescribed collection from Ecuador under the name *Puccinia andicola* Lagerh. The writer has examined a portion of Lagerheim's collection and has found that it is a specimen of *P. sana*. Although a few aecia are present they are too old to permit accurate description.

12. PUCCINIA SPHACELICOLA Jacks. & Holw. Mycologia **24**: 67. 1932.

Spermagonia amphigenous, in groups, subepidermal, globoid, chestnut-brown, 100–180 μ in diameter. Aecia amphigenous, chiefly hypophyllous, cylindric, 0.5–1.0 mm. in diameter and 1–3 mm. high, in groups up to 5 mm. long on leaf veins; peridial cells angularly oval or elliptical, yellowish, 30–40 × 34–50 μ in surface view, inner wall 9–12 μ thick, verrucose, outer wall 7–9 μ thick, finely striate; aeciospores globoid, ellipsoid, pyriform, or broadly fusiform, 23–32 × 30–42 μ; wall pale yellow or hyaline, verrucose-tessellate, 3–4 μ thick, occasionally prolonged at one end to form a papilla or short beak. Uredia unknown, probably lacking. Telia epiphyllous, scattered, round, compact, becoming pulverulent, chestnut-brown, 0.5–1.0 mm. in diameter; teliospores ellipsoid or oblong, 23–30 × 28–37 μ, rounded at both ends, not constricted at the septum; wall cinnamon-brown, paler over the pores, finely and evenly punctate-verrucose, appearing striate, about 2 μ thick, thickened to 3–4 μ over the pores and to 4–5 μ at the septum angles, pore of upper cell apical, of lower next to the pedicel; pedicel hyaline, thin-walled, fragile, breaking at or near the point of attachment, occasionally attached obliquely.

HOSTS AND DISTRIBUTION: *Salvia rumicifolia* Kunth, Ecuador.

ILLUSTRATIONS: FIG. 12 (from type).

EXSICCATI: Reliq. Holw. 426 (host listed as *Sphacele paniculata* Benth.).

Specimens examined: On *Salvia rumicifolia:* Riobamba, Ecuador, Aug. 10, 1920, Holway *865* (type); Quito, Ecuador, Aug. 23, 1920, Holway *937;* Riobamba, Ecuador, Feb. 4, 1934, Schimpff *933.*

Jackson (**5**) described *Puccinia sphacelicola* from Holway's No. *865,* collected at Riobamba, Ecuador. At that time the host was determined as *Sphacele paniculata* Benth. Later, Epling (**4**) cited Holway's No. *865* as a specimen of *Salvia rumicifolia.* The specimen cited above as Schimpff *933* is on leaves of *Salvia rumicifolia,* from the phanerogamic collections of the U. S. National Herbarium. These leaves were found to bear a few aecia and telia of *P. sphacelicola.*

13. **Puccinia striatifera** sp. nov.

Spermagoniis, aeciis et urediis adhuc ignotis. Teliis amphigenis, rotundatis, pulverulentis, castaneo-brunneis, sparsis, 0.2–1.5 mm. diam., vel in greges usque ad 4 mm. diam.; teliosporis ellipsoideis, 20–26 × 36–43(–52) μ, utrinque rotundatis, medio constrictis; membrana cinnamomeo-brunnea, uniformiter 2 μ crassa, supra poros usque ad 3 μ leniter incrassata, striata, poro superiore apicali, inferiore juxta pedicellum sito; pedicello hyalino, fragili, brevissimo.

Spermagonia, aecia and uredia unknown. Telia amphigenous, round, compact, becoming pulverulent, chestnut-brown, scattered, 0.2–1.5 mm. in diameter or in groups up to 4 mm. across; teliospores ellipsoid, 20–26 × 36–43(–52) μ, rounded at both ends, constricted at the septum; wall cinnamon-brown, uniform, about 2 μ thick, thickened slightly to about 3 μ over the pores, transversely striate; pore of upper cell apical, of lower next to the pedicel; pedicel hyaline, thin-walled, fragile, breaking away at the point of attachment.

On leaves of *Salvia revoluta* in Peru.

HOSTS AND DISTRIBUTION: *Salvia revoluta* R. & P., Peru.

TYPE LOCALITY: Huariaca, Peru, on *Salvia revoluta.*

ILLUSTRATIONS: FIG. 13 (from type).

SPECIMENS EXAMINED: On *Salvia revoluta:* Huariaca, Peru, Sept. 13, 1922, Macbride & Featherstone *2407.*

This species, of which only the telial stage is known, is based on a specimen obtained from the phanerogamic collections in the her-

barium of the Chicago Museum of Natural History. It resembles *P. sphaecelicola* in the marking of the teliospore wall and the position of the pore in the lower cell but differs in having longer teliospores which are constricted at the septum. The teliospore wall in *P. striatifera* is also much more uniform in thickness, lacking the thickening at the angles of the septum.

14. PUCCINIA IMPEDITA Mains & Holw. apud Arthur Mycologia **10**: 135. 1918.

Uredo salviarum Mayor, Mem. Soc. Neuch. Sci. Nat. **5**: 592. 1913.

Bullaria impedita Arth. & Mains, N. Am. Flora **7**: 493. 1922.

SPECIMENS EXAMINED: On *Salvia cataractarum* Briq.: Andes Orientales, Dept. Cundinamarca, Colombia, Oct. 12, 1910, Mayor *49*. On *Salvia macrostachya* Kunth: Pangor, Ecuador, Sept., 1891, Lagerheim. On *Salvia mayorii* Briq.: Andes Orientales, Dept. Cundinamarca, Colombia, Oct. 12, 1910, Mayor *47*. On *Salvia occidentalis* Sw.: Trinidad, March 3, 1921, Seaver; Moruga, Trinidad, March 19, 1921, Seaver; Huanuco, Peru, Sept. 23, 1922, Macbride & Featherstone. On *Salvia petiolaris* Kunth: Andes Centrales, Dept. Antioquia, Aug., Sept., 1910, Mayor *203* (type of *Uredo salviarum*). On *Salvia tiliaefolia* Vahl: Villa Aspiazu, Sur Yungas, Bolivia, May 28, 1920, Holway *675;* Hacienda Anacuri, Nor Yungas, Bolivia, June 2, 1920, Holway *700* (Reliq. Holw. *423*).

This species and the one immediately following occur chiefly in North America. Descriptions of them can be found in the paper by Baxter and Cummins (**2**) on the North American species of this group.

Holway's specimen No. *700* has telia as well as uredia. The teliospores are paler and thinner-walled than are those of North American specimens of this rust, but agree otherwise as do the urediospores.

Uredo salviarum Mayor was placed in synonymy with *Puccinia salviicola* Diet. & Holw. by Arthur (**1**). Later it was transferred to synonymy with *P. ballotaeflorae* Long by Kern, Thurston and Whetzel (**6**), on the basis of the number and position of the germ pores. The writer has examined Mayor's specimens of *U. sal-*

viarum and has found that this species agrees more closely with *P. impedita*. It has accordingly been transferred to synonymy with the latter species.

15. PUCCINIA FARINACEA Long, Bull. Torrey Club **29**: 115. 1902.
Puccinia prospera Arth. Bull. Torrey Club **46**: 118. 1919.
Dicaeoma prosperum Arth. N. Am. Flora **7**: 414. 1921.
Dicaeoma farinaceum Arth. N. Am. Flora *7*: 411. 1921.

SPECIMENS EXAMINED: On *Salvia rhinosina* Griseb.: Jujuy, Argentina, Feb. 15, 1939, Balls *6067*. On *Salvia stachydifolia* Benth.: Cagunilla, Bolivia, March, 1933, Cardenas *439*. On *Salvia* sp.: Vespucio, Dept. Oran, Argentina, July 14, 1937, Cabrera *4186*.

This highly variable species is widely distributed in North America, where it occurs from the central United States southward through Central America. The known range is here extended to include Bolivia and Argentina.

The collection on *Salvia* sp. from Argentina, Cabrera's No. *4186*, exhibits a phenomenon observed previously (**2**) in a specimen of *P. farinacea* from Guatemala. In addition to the typical overwintering spores the telia contain numerous pale, thin-walled teliospores which germinate without a resting period. The lability exhibited by such collections may be an indication that *P. farinacea* is tending toward the microcyclic condition.

EXCLUDED SPECIES

Puccinia griseola Lagerh, apud Syd., Monogr. Ured. **1**: 296. 1904.

This species has sessile teliospores and belongs in the genus *Polioma*, which has recently been reinstated by Baxter and Cummins (**3**) to accommodate four species having 2-celled sessile teliospores.

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LAFAYETTE, INDIANA

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2. **Baxter, J. W. and G. B. Cummins.** A monograph of species of *Puccinia* occurring on *Salvia* in North America. Lloydia **14**: 201–230. 1951 (issued March 1952).
3. —— **and** ——. *Polioma* Arth., a valid genus of the Uredinales. Bull. Torrey Club **78**: 51–55. 1951.
4. **Epling, C.** A revision of *Salvia*, subgenus *Calosphace*. Publ. Univ. Cal. L. A. in Biol. Sci. **2**: 1–383. 1940.
5. **Jackson, H. S.** The rusts of South America based on the Holway collections—VI. Mycologia **24**: 62–186. 1932.
6. **Kern, F. D., H. W. Thurston, Jr. and H. H. Whetzel.** Annotated index of the rusts of Colombia. Mycologia **25**: 448–503. 1933.
7. **Mayor, E.** Contribution á l'étude des Urédinées de Colombie. Mém. Soc. Neuch. Sci. Nat. **5**: 442–599. 1913.
8. **Sydow, P. and H.** Monographia Uredinearum 1. 972 pp. Borntraeger, Leipzig. 1904.

GERTRUDE SIMMONS BURLINGHAM
1872–1952

FRED J. SEAVER

(WITH PORTRAIT)

Dr. Gertrude S. Burlingham, well-known mycologist, passed away January 11, 1952 in her home in Winter Park, Florida, after a rather extended illness. Miss Burlingham was born in Mexico, New York, April 21, 1872, and spent all of her professional life in her native state. For many years she was a teacher of biology and grade adviser at Eastern District High School, Brooklyn. Early in her career, however, she adopted Vermont as her second home and throughout her professional life she spent her summers in her adopted state.

My first contact with Dr. Burlingham was while at the New York Botanical Garden, when on week ends this modest, quiet little woman used to wander into the herbarium and browse through the collections checking up the material collected by her during her summer vacation. How well do I recall how she showed with great pride the picture of the house she had built for herself on Newfane Hill, Vermont, in 1914. Even after her retirement to Florida in 1934 she continued to migrate with the birds to the old nesting place returning at first from California and later from Florida, where she purchased a winter home in 1936. At her request her physical remains were interred on the Hill she loved so much during her lifetime. But scientists, like "old soldiers," never die. They merely pass on but continue to live through the contributions they have left behind.

Dr. Burlingham was an outstanding authority on the genera *Russula* and *Lactarius*. Her interest, however, was not confined to these forms. She had a wide knowledge of the fungi in general and having grown up on a farm she was an all-round naturalist. In her zeal for field knowledge of the fungi she spent three seasons collecting on the Pacific coast, in Washington, Oregon and

California, and made a journey to Sweden to collect with the Swedish students of the Hymenomycetes, Lars Romell and Seth Lundell, and the Danish master, Jacob Lange. This, of course, was in addition to her constant field work along the Atlantic coast. She was a graduate of Syracuse University and took her doctor's degree in Columbia University in 1908. Much of her

GERTRUDE SIMMONS BURLINGHAM
Photograph by Tooley-Myron Studios

early work was done at the New York Botanical Garden and it is therefore quite appropriate that her extensive collections should be left to that institution, as has been done. A list of her publications, prepared by Dr. H. W. Rickett of the New York Botanical Garden follows:

Some Lactarii from Windham County, Vermont. Bull. Torrey Club **34**: 85–95. 9 Ap. 1907.

Suggestions for the study of the Lactariae. Torreya **7**: 118–123. 19 Je 1907.

A study of the influence of magnesium sulphate on the growth of seedlings. Jour. Am. Chem. Soc. **29**: 1095–1112. *f. 1–5.* Jl 1907.

A study of the Lactariae of the United States. Mem. Torrey Club **14**: 1–109. *f. 1–15.* *26* My 1908.

The Lactariae of North America—Fascicles I and II. Mycologia **2**: *27–36.* 1 Ja 1910.

The Lactarieae of the Pacific Coast. Mycologia **5**: 305–311. *25* N 1913.

[Agaricaceae.] Tribe 2. Lactarieae. N. Am. Flora **9**: 172–200. 3 F 1910. 201–236. 30 Ap. 1915.

Methods for satisfactory field work in the genus *Russula.* Mycologia **9**: *243–247.* 30 Jl 1917.

New species of *Russula* from Massachusetts. Mycologia **10**: 93–96. 4 Ap 1918.

A preliminary report on the *Russulae* of Long Island. Mem. Torrey Club **17**: 301–306. 10 Je 1918.

Some new species of *Russula.* Mycologia **13**: 129–134. *pl. 7* + *f. 1–6.* Je 1921.

Notes on species of *Russula.* Mycologia **16**: 16–23. *pl. 4, 5.* 19 Ja 1924.

Two new species of *Lactaria.* Mycologia **24**: 460–463. *f. 1–3.* 1 S 1932.

New or noteworthy species of *Russula* and *Lactaria.* Mycologia **28**: *263–267.* *f. 1–8.* 1 Je 1936.

Two new species of *Russula* together with the spore ornamentation of some of our American russulas. Mycologia **31**: 490–498. *f. 1–11.* 1 Au 1939.

Interesting species of *Lactariae* from *Florida.* Mycologia **32**: 575–586. *f. 1–4.* 1 O 1940 (with Henry Curtis Beardslee).

Spore ornamentation of some American *Russulae* and a new species of *Lactaria.* Mycologia **34**: 8–12. *f. 1–11.* 1 F 1942.

Studies in North American Russulae. Mycologia **36**: 104–120. *f. 1, 2.* 1 F 1944.

Noteworthy species of *Lepiota* and *Lactaria.* Mycologia **37**: 53–64. *f. 1–7.* 3 F 1945.

Henry Curtis Beardslee. Mycologia **40**: 505, 506. *port.* 29 S 1948.

RENÉ VANDENDRIES
1874–1952

HAROLD J. BRODIE

(WITH PORTRAIT)

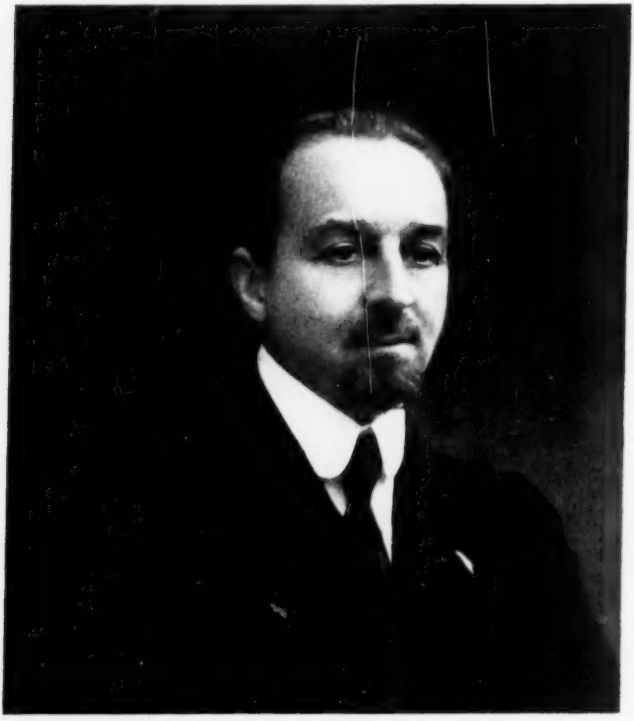
Mycologists in America join their colleagues in Europe and elsewhere in marking with sorrow the passing on March 28, 1952, of the renowned Belgian savant Dr. René Vandendries at his villa in Rixensart, Belgium.

In a modest laboratory attached to his home this man, single-handed, contributed a monumental volume to the knowledge of sexuality among basidiomycetous fungi. His name is associated forever with the names of Bensaude, Kniep, Buller and others who were responsible for revealing the unique characteristics of sexuality in the higher basidiomycetes. Vandendries' contribution to this "realm," as he called it, is two-fold: he analyzed the mating behavior of mycelia of many species and he carried out extensive and detailed studies of races of these fungi to determine the distribution of genes governing sexuality not only in large numbers of populations of European origin but also in populations from America.

Study of the subject of multiple-allelomorph heterothallism in the fungi as reviewed in 1949 by Whitehouse (see below), shows clearly that Vandendries' methodical research on fifty-five different species of Hymenomycetes was no mere cataloguing of their patterns of sexual behavior. From data of this kind Whitehouse and others have been able to present a remarkably full account of the distribution of homothallism and of bipolar and tetrapolar heterothallism among the Hymenomycetes and to state important generalizations as to the number of alleles at the loci for heterothallism in natural populations of the fungi. When it is realized that the analysis of each species or each race represents, on the average, the results of some three hundred pairings between single-spore

mycelia, it becomes evident that students of genetics and sexuality of the fungi owe much to Vandendries' work.

Vandendries was born in Louvain in 1874, the son of Flemish parents. At the University of Louvain he studied botany under the tutelage of Carnoy and there obtained the degree Doctor of



RENÉ VANDENDRIES AS A YOUNG MAN

Natural Sciences in 1898. Later he became a professor of the Athenée Royale of Antwerp and he taught also at Bruges and elsewhere. Upon retirement from university life in the late 1920's, Dr. Vandendries became an Inspector of those schools in Belgium which would be designated in America as normal schools or teachers' colleges. In whatever professional capacity he served, Vandendries was eminently successful: as a teacher he was lucid

in exposition and exacting in requirements; as an inspector he is remembered as a severe critic but a kindly judge.

Many honors were bestowed upon Dr. Vandendries by his country and by learned societies. Among these may be mentioned: Commander of the Order of Leopold, Grand Officer of the Order of the Crown of Belgium, Member of the Royal Thérésian Academy of Belgium and Member of the Royal Botanical Society of Belgium. The Botanical Society of America elected him Corresponding Member in 1947.

Dr. Vandendries married Anne Virginie Frélinckx who predeceased him in 1948. He is survived by one son, Henry, of the Evangelical Mission of Brussels, and a nephew, Dr. Corneille Vandendries, a chemist.

Prior to World War II, Vandendries went to live in Portugal because of his wife's poor health. There for several years the couple lived under conditions almost of privation owing to isolation from their native land and to the inaccessibility of their pension income. Scientific work was impossible for Vandendries during this time, but it was characteristic of him not to remain idle. Despite the burden of his wife's illness and his own failing strength, he began the writing of a long series of personal narratives under the title of "Contes et Souvenirs." Six volumes of these in longhand remain unpublished. The delightful stories are drawn from his own experiences and reveal his true character. The pages are filled with keen observations of nature, with fine humor and with a deep appreciation of beauty. He recounts his experiences at the International Genetics Congress in Ithaca which he attended by invitation in 1932; he reveals the pleasure derived from the visit to his laboratory of the late Professor T. H. Morgan; he tells amusing anecdotes about collecting and studying fungi. His stories of dogs and cats he has "known" remind one of La Fontaine, so beautifully and entertainingly are they written.

His students remember Vandendries as a short rather dapper man with an aristocratic bearing whose paramount personal characteristics were kindliness and good humor. To be received into his happy household not only as a student but almost as a son, was an experience of incalculable value. One was expected to put in long hours in the laboratory, but on Sundays there were walks

through the woods from Brussels to Wavre. On these occasions Vandendries would throw great energy and enthusiasm into hunting for fungi but would stop to examine and comment upon anything of special interest from geological formations to flowering plants and from Flemish pastry shops to gems of architecture. Despite his broad interests, his knowledge was amazingly profound. At other times there was music: Vandendries was an accomplished violist and Madame had at one time sung in the Brussels opera. His last admonition to the department student was usually: "No matter what you accomplish, your greatest reward will be the joy of the work itself."

Reference to over fifty of Vandendries' most important publications may be found in the articles which are listed below.

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DEPARTMENT OF BOTANY,
INDIANA UNIVERSITY,
BLOOMINGTON, INDIANA

NOTES AND BRIEF ARTICLES

Pigment Study of Dacrymyces Ellisii

Basidiospores of *Dacrymyces Ellisii* were germinated on malt agar. Bright yellow colonies 0.5–4 cm. in diameter resulted. After thirty days in the light at room temperature the colonies were manually removed from the agar. The collected colonies were homogenized and extracted with acetone. The acetone extract was evaporated, the pigments taken up in petroleum ether and, after saponification, chromatographed. Ten pigments were isolated.

TABLE I

a. Column 30 cm. long, 2 cm. diameter and packed with Alcoa activated alumina grade F-20. b. Absorption maxima in petroleum ether B. P. 30–60° C. c. Amounts of pigment present in 7 gms. dry weight of colonies after extraction with acetone. Amounts estimated from the absorption curves. d. All fractions give a positive test for carotenoids with concentrated sulfuric acid. e. Point of inflection. f. Tentatively identified from absorption spectra and color tests. g. Isolated from the hypophase, after saponification with 10% methanolic potassium hydroxide and hydrochloric acid, and chromatographed separately.

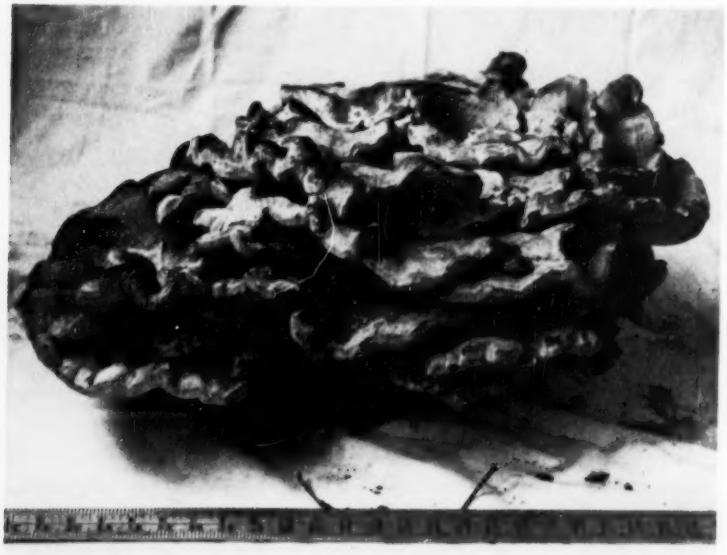
CAROTENOIDS FROM DACRYMYCES ELLISII

BAND TOP DOWN	BAND COLOR	BAND WIDTH (cm.) [a]	ABSORPTION MAXIMA [b]			ESTD. AMTS. (mg.) [c]	PIGMENT	PARTITION (%) [d] 95% CH_3OH	90%
1	YELLOW	0 - 0.2	424			0.01	I	0	0
2	ORANGE	0.6-0.8	400 [e]	444	477	0.1	II	10	0
3	PALE PEACH	1.0-2.8	420	450 [e]		0.014	III	30	0
4	DEEP PEACH	2.8 - 4.0	443			0.05	IV	15	0
5	PALE ROSE	4.2-8.2	424	448 [e]		0.005	V	-	-
6	PALE ROSE	8.8-23	446	473	501	0.009	LYCOPENE [f]	TRACE	0
7	LT YELLOW	24 - 26	430	463	495	0.025	γ-CAROTENE [f]	TRACE	TRACE
8	YELLOW	27 - 30-	427 [e]	452	483	2.5	β-CAROTENE	TRACE	TRACE
9 [g]	ORANGE	1.2 - 1.4	-			0.015	H I	TRACE	0
10 [g]	YELLOW	1.4 - 3.0	-			0.004	H II	TRACE	0

By far the major pigment was β-carotene (TABLE I).—CALVIN HANNA AND THOMAS J. BULAT, Departments of Pharmacology and Botany, State University of Iowa.

A LARGE SPECIMEN OF POLYPORUS BERKELEYI

The extremely large size of the fruiting bodies produced by certain puffballs and polypores is always of interest. *Polyporus Berkeleyi* Fries is reputed to be the largest species of *Polyporus* found in the United States, and recently an unusually large fruc-



POLYPORUS BERKELEYI

tification of this species was collected by Mr. H. H. Wiebe and the author. The fungus was fruiting at the base of a large white oak in the Duke University Forest, Orange County, North Carolina, and when collected on July 20, 1952, the fructification was found to weigh 45 pounds. It was roughly circular in outline and measured from 23 to 26 inches (58 to 66 cm.) in diameter, with a height of 13 inches (33 cm.). The accompanying photograph shows the many imbricated pilei making up the fruiting body.

Other large fructifications of this species reported in the literature include the one Lloyd (Mycol. Notes, Polyporoid Issue, **3**: 36. 1910) received from Trenton, New Jersey, which weighed 43 pounds and measured 26.5 by 36 inches (67 by 91 cm.), with a height of 13 inches (33 cm.), and the one McIlvaine (One Thousand American Fungi) saw in Boston, which was 4 feet (1.2 m.) tall and 2.5 to 3 feet (76 to 91 cm.) wide. Since this species is commonly pictured with a very long underground stem, there is no way of knowing the relative proportion of stem to fruiting surface of the Boston specimen, and the weight was not given.—W. H. WILLS, Duke University.

NUTRITION OF MORCHELLA

In a paper published by the author (Mycologia **43**: 402–422. 1952) on the nutrition of *Morchella esculenta*, it was stated that little work had been done on the physiology of *Morchella*. Since that time it has been discovered that a paper published by Hans Hurni (The physiology of *Morchella esculenta*. Its synthesis of aneurine under various conditions. Mitt. naturforsch. Ges. Bern (N.S.) **4**: 11–18. 1947. (See also Chem. Absts. **45**: 9140) has covered many of the points that the present writer's paper investigated. Where similar things have been done, the results agree very closely.

It is interesting that two strains of *M. esculenta*, isolated from different parts of the world, should give the same nutritional responses. In a non-sporulating, non-fruiting mycelial form like *Morchella*, there is a paucity of morphological characteristics suitable for taxonomy, and it may be that nutritional responses may serve as an adequate adjunct to the usual methods.—THOMAS D. BROCK, Research Laboratories, The Upjohn Company.

THE NEW RULES

The International Code of Botanical Nomenclature (the title of what amounts to the fourth edition of the Rules) arrived early in November. Copies can be secured by those not members of the International Association for Plant Taxonomy for $3.70 (paper)

or $4.20 (buckram) through Dr. Reed C. Rollins, Gray Herbarium, 79 Garden Street, Cambridge 38, Massachusetts.

A NEW JAPANESE MYCOLOGICAL JOURNAL

Volume 1, No. 1 of NAGAOA, mycological journal of the Nagao Institute, 6–387, Kitashinagawa, Tokyo, Japan, has recently appeared. A preface and introductory outline indicate that the Institute is a private foundation for research in industrial mycology. NAGAOA is announced as biannual; the first number includes two papers of a series of "Enumerations of the Moulds of Japan," a first report of "Studies of the Sporobolomycetaceae in Japan" (all in English), an article "On the Genus *Graphiola* found in Japan" (Japanese with Latin diagnosis), and a forty-page "List of Cultures." The subscription price is $1.00 a year.

REVIEWS

THE YEASTS. A TAXONOMIC STUDY, by J. Lodder and J. J. W. Kreger-van Rij. xi + 713 pp. 268 figs. North Holland Publishing Co., Amsterdam; Interscience Publishers, Inc., New York. 1952. Price $19.50.

This remarkable compendium of all the yeasts, in one volume, provides an invaluable, authoritative guide and research tool for all who have any occasion to consider the taxonomy or nomenclature of these fungi. Although based upon the three volumes in which the authors have monographed the yeasts over a 20-year period, the book is entirely new. Modifications and additions over previous taxonomic works have been based upon and largely necessitated by modern advances, especially in our knowledge of the biology, genetics, and physiology of yeasts. The authors have been eminently qualified in this tremendous task not only in their own research experience but in the facilities at their command. At the Koninklÿke Nederlandsche Gisten Spiritusfabriek, and the Yeast Division of the Centraalbureau voor Schimmelculturs, both at Delft, there has been assembled one of the most extensive collections of yeasts available anywhere in the world.

The term yeast is used by the authors in its broad sense to include not only the ascosporogenous family, the Endomycetaceae, and the imperfect family, the Cryptococcaceae, but also the yeast-like fungi characterized by ballistospores and classified usually in the Sporobolomycetaceae. Some may question the propriety of including the Sporobolomycetaceae in a treatment of the yeasts. Certainly, however, its inclusion increases the utility of the book as a guide in determining the identity of any yeast-like organism.

In addition to its seven chapters, a section in the back of the book is devoted to Latin diagnoses of 15 new species, 4 new varieties, and 1 new genus. An author and a subject index are both provided as well as a list of references at the end of the chapters. The subjects taken up in each chapter are as follows: I. Intro-

duction. II. Characteristics and methods used in the classification. III. Variation in yeasts and its significance in taxonomy. IV. General classification of the yeasts. V. Discussion of the genera belonging to the Endomycetaceae. VI. Discussion of the genera Sporobolomyces and Bullera. VII. Discussion of the genera belonging to the Cryptococcaceae.

The initial division of the yeasts into three families is a morphological one, based on the formation of ascospores, ballistospores, or the lack of either. Genera, as well as the families, are also based essentially on morphological characters pertaining to vegetative and sexual reproduction. Species and subspecies groups, however, are established for the most part on physiological properties. This is in general accord with the views of other systematists working with yeasts and derives from a conviction that in practice it is impossible to base a system of yeast taxonomy on morphological characters alone. It is not a view, however, that will be accepted wholly by those mycologists who adhere to the morphological concept of species championed repeatedly by the International Congresses. On this basis, the yeast species are in effect physiologic races and the genera, morphologic species.

Foremost among the physiological tests used by the authors in separating species is the ability to ferment sugars with the formation of ethanol and carbon dioxide, employing such sugars as glucose, mannose, fructose, galactose, saccharose, maltose, lactose, raffinose and inulin. For the non-fermenting yeasts the property of using sugars as an assimilation and a respiration substrate has been the criterion in separating species. Other physiological properties, to be sure, have also been made use of, such as the assimilation of nitrogen, other carbon and sulfur compounds, amino acids and the vitamins.

One of the interesting features of the book is the analysis made of the different morphological and physiological properties of yeasts from the standpoint of their suitability as taxonomic criteria. The result has been the establishment of clearly set forth methods, carefully systematized and described, even to the details of the formulation and the preparation of media. In general, the yeasts were maintained on malt agar, and incubated at about 25° C. Purity was generally ascertained by the selection of single colonies from

dilution plates. Single cell transfers were resorted to in special cases.

Variation in yeasts was found by analysis to be largely of 3 types: changes in the genotype, cytoplasmic changes, and segregations. The genotype changes were considered to be hereditary and to have arisen by mutation in contrast to cytoplasmic changes which arise in the plasma or structural elements other than the nucleus. The changes from rough to smooth, likened to the S-R phenomenon in bacteria, accompanied by degeneration and loss of sporulation, are attributed to mutation but are reported to be reversible in some cases, stable in others. Those who have worked with mutations in other fungi may well question whether the mutations in yeasts are truly reversible when analyzed by the single cell culture method or, indeed, whether the changes referred to as reversible mutations are in fact true mutations.

Although remarkable changes in morphology or colony characters were attributed to mutation, these changes were generally accompanied by relatively slight changes in physiological characters, i.e., fermentative powers, the changes being mostly of a quantitative, seldom of a qualitative nature. The conclusion that physiological characters in the yeasts, more especially the fermentative properties, are fairly stable in spite of mutations strongly supports the use of such properties in the delimitation of their species or, for what mycologists in general would term, their races. Finally, after a discussion of the changes due to segregations, the authors find themselves neither in accord with Winge's "strictly orthodox Mendelian interpretation" nor with Lindegren's "rather revolutionary and confusing theory" in explanation of the various observations which have been made of segregation changes.

Apparently the facts of variation are found "rather depressive" for the yeast systematist. The problem is how to take into account in taxonomy the changes of one kind or another which are continually recurring and which, it is understandingly claimed, are not guaranteed against by starting with a single yeast cell. The cause of this dilemma, as seen by the authors, is that the morphological characteristics valued so highly in botanical taxonomy are very susceptible to all three types of variation. But is it not the real point that most botanists would not attempt to differentiate races

on morphological grounds? Yeast species are the horticultural varieties of the botanist.

With this introduction of the problems in yeast taxonomy and an explanation of the current species concept in yeasts, the authors launch into the actual systemics of yeasts in Chapter IV, with simply constructed keys for the separation of families and genera. Some 263 pages are devoted to the Endomycetaceae which includes keys, descriptions, diagnoses, historical surveys, synonyms, taxonomic discussions, often line drawings, and summaries and literature lists. The Sporobolomycetaceae take up 28 pages and the Cryptococcaceae 296 pages.

A total of 25 genera, 165 species and 18 varieties are recognized in this book, if we have added correctly. It is a refreshing experience to find the emphasis in a taxonomic treatment of fungi placed on synonymy rather than on the creation of new species.

Written in English of great clarity, simplicity, and directness, the book is designed in such a form as to be readily used by any biologist interested in yeasts. It is indispensable to the zymologist and yeast technologist. Every mycologist would want it as a reference book. What better tribute could a mycologist pay to this excellent work than to hope some day that other groups of the fungi may be treated with similar lucidity and precision.—WILLIAM C. SNYDER.

DISEASE IN PLANTS. An Introduction to Agricultural Phytopathology, by Neil E. Stevens and Russell B. Stevens. xx + 219 pp., 61 figs. plus additional cuts. Waltham, Mass., The Chronica Botanica Co., New York City: Stechert-Hafner, Inc. 1952. Price $4.75. Volume one of Chronica Botanica's "Manual of the Plant Sciences."

This is a unique and provocative book, well organized, very readable, clearly printed, and unusually free from mechanical errors. The book differs materially from other texts in plant pathology as details are omitted on specific diseases and techniques. Instead, the authors discuss plant pathology on the basis of broad, general principles. The book awakens the readers to the intimate relationship between plant pathology and related biological fields, but sometimes the related subject could be more effectively inte-

grated. Because of the general scope of the book, some may question its value as an elementary text, unless it is used in conjunction with a standard text in plant pathology.

The volume consists of twenty chapters covering a wide range of topics. Except for the first two chapters, they are grouped under three main headings: causal agents of plant diseases, factors influencing the development of pathogenic diseases, and disease control. In most cases only a small number of references are cited at the end of each chapter. Hence, the value of the book, particularly as a reference, could be materially increased, provided more original work were cited. The 61 illustrations, many of which have been published before, are in general of high quality; the volume also includes several cuts of historical interest. Although the book is intended primarily as an elementary text, it has even greater value for general professional and cultural reading.—J. J. CHRISTENSEN.

INTRODUCTORY MYCOLOGY, by C. J. Alexopoulos. 482 pp., 187 figs. John Wiley & Sons. 1952. Price, $7.00.

The author of this text-book of general mycology has certainly achieved his primary aim, which is to present an adequate treatment of the subject for a one-term course in mycology. For an introductory text, the book contains an unusual quantity of important details. With the addition of supplementary reading assignments, such as the references listed at the end of each chapter, the book could also be adapted for use in a two-term course. One of the most outstanding features of the book is the large number of illustrations, particularly the numerous life cycles illustrated with drawings selected from various research papers. It is noteworthy that the student is made aware of the more outstanding recent trends in the field of mycology. The writing is clear and not too technical, and the derivations of most of the technical terms are included. Also there is a very useful glossary of mycological terms.

The system of classification followed by Dr. Alexopoulos is fairly conventional and demonstrates his cognizance of recent trends in taxonomic research. It is commendable that the author has seen fit to include a chapter on the bacteria and actinomycetes, particu-

larly in view of the fact that it is becoming increasingly difficult to draw a sharp line of demarcation between these organisms and the fungi. Also included are the slime molds. The Fungi Imperfecti, an important group of organisms frequently neglected in mycological texts, are given adequate treatment. The Phycomycetes are placed in three main series—Uniflagellatae, Biflagellatae, and Aplanatae—with emphasis upon flagella, as advocated by Sparrow and by Karling. The Ascomycetes are treated in two sub-classes: the Protoascomycetes, including the Endomycetales and Taphrinales, and the Euascomycetes, including the Aspergillales, Myriangiales, Erysiphales, Laboulbeniales, Sphaeriales, Hypocreales, Microthyriales, Dothidiales, Pseudosphaeriales, Hysteriales, Ostropales, Helotiales, Pezizales, and Tuberales. The Erysiphales are placed in the Pyrenomycetes, rather than in the Plectomycetes, because of the regular arrangement of asci in the cleistothecium. The Mycosphaerellaceae and Pleosporaceae are included in the Pseudophaeriales, and the Phyllachoraceae are discussed under the Sphaeriales. The Basidiomycetes are divided into the two subclasses: Hemibasidiomycetes, including the Dacrymycetales, Tremellales, Auriculariales, Uredinales, and Ustilaginales, and the Holobasidiomycetes, which are divided into the conventional two series, Hymenomycetes and Gasteromycetes. All seven families of Hymenomycetes are placed in the single order, Agaricales, while the Gasteromycetes are divided into five orders. Throughout the text there are keys to the major groups (not including genera) of fungi.

There are some mistakes, most of which are not of a very serious nature. On more than one occasion it is stated that meiosis occurs in the first division of the zygote nucleus; whereas, two divisions are required for the completion of this process. If all of Blakeslee's investigations on sexual reproduction in the mucors are taken into consideration, it is very unlikely that one can conclude that Blakeslee regarded heterothallism as representing a true segregation of "sexes" in different thalli. Furthermore, it appears that most writers of mycological texts are unaware of a paper by Köhler, published in 1935, and one by Sjöwall, which appeared in 1946, that offer an explanation for the occurrence of sporangiospores of only one compatibility type in a single zygosporangium

of certain heterothallic mucors. Also, the coenzygotes of *Rhizopus nigricans* have now been germinated (by Sjöwall) and the life cycle has been demonstrated to resemble that of *Mucor mucedo*. In the discussion of the yeasts, it is unfortunate that the old concept of Wager and Peniston regarding the structure of the yeast nucleus is perpetuated, in view of the overwhelming recent evidence against it. It is erroneously stated that the heterokaryotic condition in *Neurospora sitophila*, with respect to mating types, may arise normally through hyphal fusions, and there are some discrepancies in the description of aeciospore formation in the rusts.

However, the mention of these errors should not be allowed to detract from the overall value of the book. It is a text which has long been needed for the teaching of a one-term course in mycology, and it has many important features which make it useful to more advanced students as well.—L. S. OLIVE.

LABORATORY MANUAL FOR INTRODUCTORY MYCOLOGY, by C. J. Alexopoulos and E. S. Beneke. 177 pp., 95 figs. Burgess Publishing Co., Minneapolis. 1952. Price, $3.25.

While primarily intended to be used in connection with the Alexopoulos or the Bessey text, this is suitable for general use. A brief introduction discusses preparation of temporary and permanent mounts, collection and preservation of fungi and culture methods. This is followed by a concise outline of the classification adopted, the principal divisions recognized being the bacteria, the slime molds and the true fungi. The exercises are not numbered and since the genera mentioned are for the most part those which are common and available in temperate regions generally, the instructor may use discreet selection of material to be studied. Each section is followed by a brief glossary and a selected list of special references. Few students will study all the genera mentioned but any who study a fair sample cannot fail to acquire a basic knowledge of the significant features of the major groups of fungi and an impression of the wide variability of the organisms included in that category.—G. W. M.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to members of the Mycological Society of America. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

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